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CHRONIC PULMONARY SCHISTOSOMIASIS[1,2]

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INTRODUCTION

Three particular species of schistosome worms are known to be human parasites. The geographic distribution of each is specific for the specie. *Schistosoma haematobium* is found in Africa and in parts of Western Asia. *Schistosoma mansoni* is present in Africa and South America. *Schistosoma japonicum* is restricted to the Far Eastern countries. Pulmonary schistosomiasis has been reported from Egypt (1–11), South and Central Africa (12–19), Jerusalem (20), Iraq (21), Venezuela (22–28), Brazil (29–36), and Puerto Rico (37–43).

The incidence of pulmonary schistosomiasis has been reported by various writers (table 1). Shaw and Abou Ghareeb (3) demonstrated that in 282 autopsies of cases of schistosomiasis occurring in Egypt, pulmonary involvement was found in 33 per cent. Of these, 2.1 per cent had definite schistosomal cor pulmonale. De Faria (35) reported from Brazil that in 5.5 per cent of 180 autopsies definite schistosomal cor pulmonale was found.

The purpose of this clinical study is to present the pathology and clinical picture of chronic pulmonary schistosomiasis and to record the pulmonary function changes and the deranged hemodynamics caused by the parasite.

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OBSERVATIONS

Pathogenesis and Pathology

The schistosoma ova, but rarely the adult worms, reach the pulmonary circulation from the veins of the bladder and intestine via the internal iliac veins and the portocaval anastomosis, respectively. On reaching the pulmonary circulation as emboli, the ova obstruct the pulmonary arterioles. The egg, approximately 110 to 170 by 40 to 70 micra in size, is mechanically hindered from reaching the capillaries. The egg makes its way through the intima, media, and adventitia of the arteriole and comes to lie immediately outside the arteriolar wall. There it initiates a tissue reaction which ultimately results in the formation of a granuloma (figure 1). Microscopically, the granuloma contains the egg or its remnants surrounded by histiocytes, eosinophilic leukocytes, lymphocytes, and multinucleated giant cells. Eventually, fibroblasts invade the granuloma, producing a nodular scar (figure 2). The two types of lesion thus produced by the ova in the lungs are designated as vascular or parenchymatous.

The vascular lesions result from passage of ova through the arteriolar walls, with resultant inflammation and necrosis. Resolution and healing of this inflammatory process leads to narrowing, thickening, and partial or complete occlusion of the pulmonary arteriole (figure 3). Later, angiomatoids may develop from canalization of the fibrous tissue obstructing the vessel (figure 4). When such vascular lesions become widespread, a diffuse endarteritis obliterans results. A definite restriction in the capacity of the pulmonary vascular bed occurs and, as a consequence, the pul-

TABLE 1

INCIDENCE OF PULMONARY INVOLVEMENT IN SCHISTOSOMIASIS AND OF SCHISTOSOMAL COR PULMONALE REPORTED BY VARIOUS WRITERS

Author	Year	Location	Number of Necropsies	Incidence	*S. mansoni*	*S. hematobium*	Both
A. Incidence of Pulmonary Involvement in Schistosomiasis							
				per cent	*per cent*	*per cent*	*per cent*
Turner (12)	1908	South Africa	59	64.4	—	100.0	—
Turner (14)	1909	South Africa	40	52.5	—	100.0	—
Shaw and Ghareeb (3)	1938	Egypt	282	33.0	30.6	58.3	11.1
Jaffe (28)	1944	Venezuela	50	24.0	100.0	—	—
Koppisch (40)	1946	Puerto Rico	126	12.8	100.0	—	—
Gelfand (17)	1950	South Central Africa	108	59.0	—	93.75	6.25
B. Incidence of Schistosomal Cor Pulmonale							
Shaw and Ghareeb (3)	1938	Egypt	282	2.1			
De Faria (35)	1954	Brazil	180	5.5			

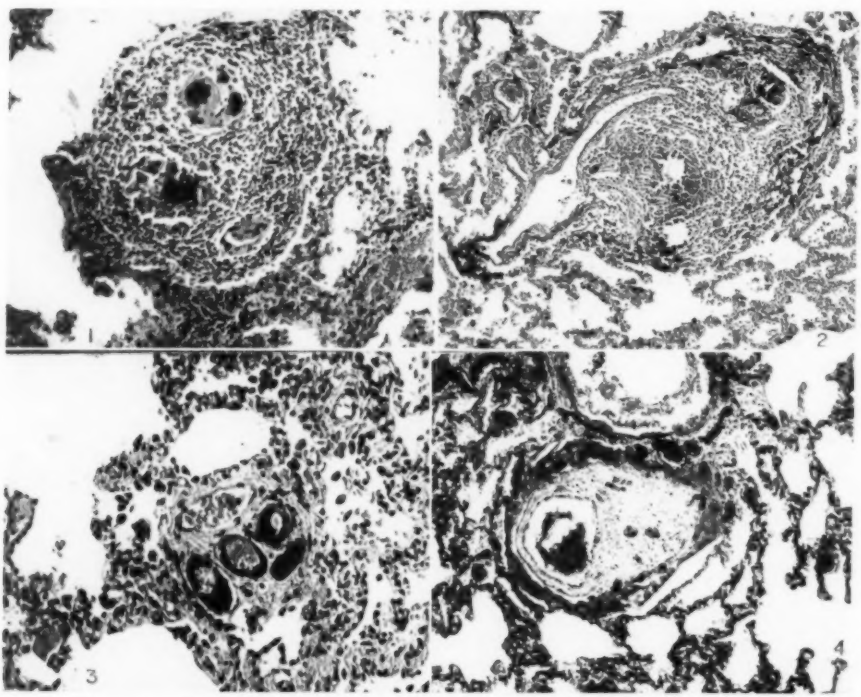

FIG. 1. "Parenchymal" schistosome granuloma with foreign-body giant cells around remnants of ova. (Hematoxylin and eosin stain, low, dry magnification)

FIG. 2. Schistosome granuloma in wall of pulmonary arteriole. Chitinous fragments of ova are seen in the center. (Verhoff-van Gieson stain, low, dry magnification)

FIG. 3. Four calcified schistosome ova impacted in fibrous tissue in wall of pulmonary arteriole. Note eccentric narrowed lumen. (Hematoxylin and eosin stain, high, dry magnification)

FIG. 4. Marked endarteritis of pulmonary arteriole, with eccentric narrowed lumen and recanalization of the scar tissue. The faint outline of a schistosome ovum can be made out in the lower right segment of the vessel. (Mallory connective tissue stain, low, dry magnification)

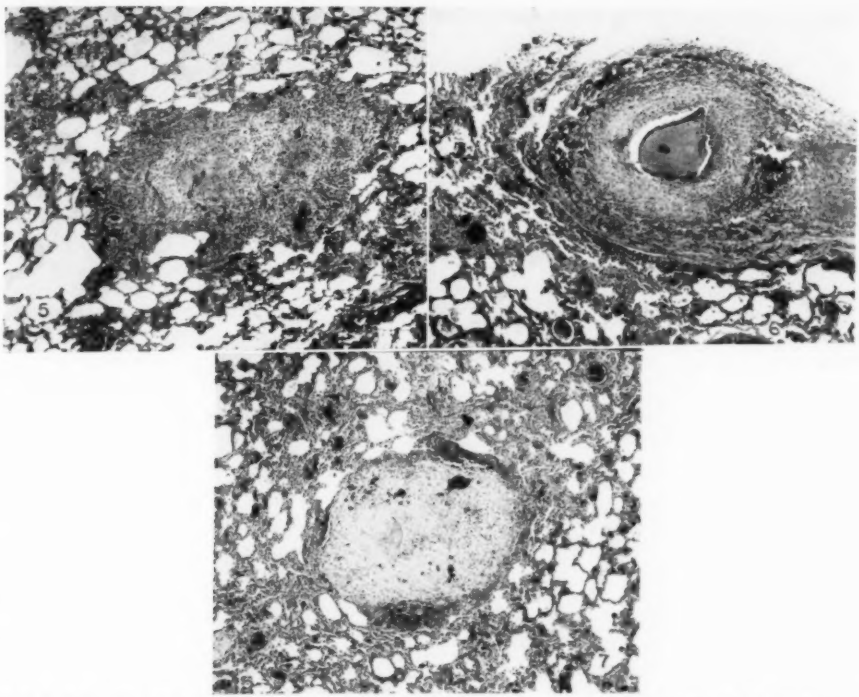

FIG. 5. Large parenchymal scar produced by healed granulomas around arteriole. Note remnants of elastica of vessels and fragments of ova. (Verhoff-van Gieson stain, scanning power)

FIG. 6. Thrombosis, panarteritis, and periarteritis in large arteriole, excited by dead worms which are not seen in this view. (Hematoxylin and eosin stain, scanning power)

FIG. 7. Focal scar in large pulmonary arteriole around dead worms. Dark spots are granules of anthracotic pigment. Note faint remains of two worms in center. (Hematoxylin and eosin stain, scanning power)

monary pressure rises. The pulmonary artery dilates and may reach aneurysmal size. The right ventricle of the heart may fail following dilatation and hypertrophy. Secondary superimposed arteriosclerotic changes in the wall of the pulmonary artery may ensue.

The parenchymatous lesions are the extravascular granulomas already described. These lie in close relationship to the bronchioles and alveoli, but in almost every case can be traced to their arteriolar origin. Such lesions are usually few in number and do not significantly alter the finer structure of the lungs. However, if a large number of granulomas result, extensive parenchymal scarring may occur (figure 5).

Pulmonary vascular lesions are rarely produced by the actual schistosome worm. The living worm excites no reaction. Dead worms produce a panarteritis, thrombosis, and occlusion of the arterial lumen. There is pneumonic consolidation of the lung parenchyma surrounding the dead worms (figure 6). Fibrosis and calcification within the vessel are the ultimate fate of the intra-arterial lesions (figure 7).

The Clinical Picture

The advanced clinical picture of schistosomal cor pulmonale has recently been described by Kenawy (8), Girgis (9), Rodriquez and Rivera (42), and Marchand and associates (43). The following description is based on the study of 15 patients examined at the Naval Medical Research Unit #3 in Cairo, Egypt (table 2).

All 15 patients were farmers and originally

lived in the Delta region of the Nile Valley. Two were females and 13 were males. Their ages ranged from seventeen to forty-five years, the average age being thirty-four years. They invariably gave a history of past schistosomal infection. All but 3 had been treated at least once with intravenous tartar emetic.

Symptoms: Dyspnea and palpitation were

TABLE 2

THE CLINICAL AND LABORATORY DATA OF FIFTEEN CASES OF SCHISTOSOMAL COR PULMONALE

	Case Number														
	1	2	3	4	5	8	9	10	11	12	15	16	17	18	19
Sex and age	M45	F50	M45	M34	M17	F28	M34	M30	M20	M40	M26	M50	M33	M22	M25
Duration of illness	5 years	3 years	6 years	1 year	1 year	6 months	6 months	2 years	4 years	6 years	3 years	9 years	7 months	5 years	1 year
Previous therapy	+	+	None	+	+	None	+	+	+	+	+	+	+	+	None
Symptoms:															
Exertional dyspnea and palpitation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Weakness and dizziness			+			+	+	+		+	+	+			+
Cough	+	+	+		+	+	+	+				+	+	+	
Orthopnea	+											+		+	
Hemoptysis				+											
Dysuria and hematuria					+	+	+		+	+	+	+		+	+
Diarrhea									+			+			
Signs:															
Increased A-P diameter of thorax	+	+				+	+	+				+		+	+
Epigastric pulsations and parasternal heave	+		+	+	+	+	+		+	+	+	+	+	+	
Hyperresonant thorax and rhonchi	+	+	+			+	+	+				+		+	+
Accentuated split second pulmonic sound	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal systolic murmur	+	+	+	+	+		+	+	+	+	+	+	+	+	
Basal diastolic murmur		+		+	+				+		+				
Hepatosplenomegaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dependent edema		+	+						+			+			
Ascites			+												
Cyanosis						+			+						
Laboratory studies:															
Stools															
S. mansoni ova	+	+	+		+	+	+	+	+	+	+	+		+	+
S. haematobium ova			+		+		+	+	+		+			+	
Urine															
S. mansoni ova			+												
S. haematobium ova				+	+				+					+	
Sputum															
S. mansoni ova			+					+		+		+			
S. haematobium ova				+											
Blood															
Hemoglobin, gm./100 ml.	14	11	12	12	13	14	12	13	14	12	13	12	13	7	11
Eosinophil per cent	8	22	4	17	16	8	20	8	33	9	20	4	3	30	14
Electrocardiogram															
Right bundle branch block			+						+			+			
Right axis deviation and right ventricular hypertrophy		+	+	+	+	+	+	+	+	+	+		+	+	+
Roentgenogram															
Dilatation of pulmonary conus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = present. Blank space = not present. A-P = anteroposterior.

Note: All of the 15 patients originally lived in lower Egypt. They all worked as farmers. Sixty per cent of the inhabitants in this area are infected with *Schistosoma haematobium* and an equal number with *Schistosoma mansoni*. (Manson-Bahr, *Manson's Tropical Diseases*, 14th edition, Williams and Wilkins Company, Baltimore, Maryland).

presenting complaints in the 15 patients. Dyspnea, increasing gradually in severity over a period of months or years, was the most prominent and disabling symptom in every case. Ten patients complained of cough with slight sputum production. One patient gave a past history of hemoptysis. Eight patients complained of weakness and dizziness. Only 3 had mild orthopnea. Acute anginal-like chest pain was characteristically absent. However, a mild precordial discomfort was experienced by one patient. Symptoms referable to urinary schistosomiasis, such as dysuria and terminal hematuria, were noted in 9 patients. Only 2 patients complained of attacks of diarrhea accompanied by blood, pus, and mucus in the stools.

Physical findings: The characteristic physical findings were related to an elevated pulmonary arterial pressure (44). An accentuated split-second pulmonary sound, parasternal heaving, and epigastric pulsations were present in nearly every case. A harsh basal systolic murmur, heard best over the second and third interspaces along the left sternal border, was present in 13 patients. Five of these patients had a basal diastolic murmur over the same area. No murmurs were heard in 2 patients. A firm nontender enlarged liver and splenomegaly were present in every patient.

In 8 patients a slight increase in the anteroposterior diameter of the thorax was noted and percussion revealed minimal hyperresonance. Auscultation of the chest demonstrated the presence of rhonchi in 7 patients. Dependent edema was present in 4 patients and ascites in one patient. Cyanosis was observed in only 2 patients. Both patients were in congestive heart failure when first examined, and the cyanosis disappeared when the failure was treated.

Laboratory studies: Twelve patients had an eosinophilia (16) ranging from 8 to 33 per cent. Only one patient had severe anemia. In 13 patients the stools were found positive for ova of *Schistosoma mansoni*, and in 4 of these patients the ova were demonstrated in the sputum (45–47). Seven of these 12 patients were also infected with *Schistosoma haematobium*, the ova being demonstrated in the urine or stools. The ova of *Schistosoma haematobium* were present in both the urine and sputum of one patient. In one patient, treated six months before with a full course of tartar emetic, the ova of neither

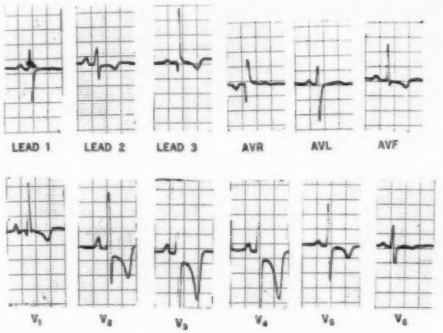

FIG. 8. *Case 4.* Electrocardiogram showing right ventricular hypertrophy and strain.

Schistosoma mansoni nor *haematobium* could be found in urine, stools, or sputum.

Electrocardiographic findings: In 12 patients the electrocardiogram was typical of right-axis deviation and right ventricular hypertrophy (figure 8). One patient had a pattern of both left and right ventricular hypertrophy. The electrocardiogram was normal in only one patient. Conduction defects were not common. A right bundle branch block was noted in only 3 cases.

Roentgenography: The roentgenographic appearance of advanced pulmonary schistosomiasis is very characteristic (48, 49). Increased dilatation of the pulmonary artery and its main branches occurs. The pulmonary outflow tract may reach aneurysmal size, and the right main pulmonary artery may dilate and appear as a comma-shaped density in the right hilar region (figures 9, 10, and 11). Fluoroscopically, the dilated right pulmonary artery has a characteristic pulsatile downward thrust. No hilar dance is evident. Occasionally a fine mottling of the lung parenchyma may be observed (figure 12).

Demonstration of ova in sputum: Detection of schistosome ova in the sputum (figure 13), combined with the above clinical picture, helps in diagnosing this disease. However, it is usually difficult to demonstrate ova in sputum specimens. The physician must often be satisfied with detecting ova in the urine and/or stool or merely with a past history of long-standing visceral schistosomiasis (16).

Diagnosis and treatment: The roentgenographic pulmonary findings are extremely helpful and should not be confused with mitral stenosis or

Case 4

FIG. 9. Postero-anterior chest roentgenogram. Note aneurysmal dilatation of pulmonary outflow tract and the dilated right main pulmonary artery.

FIG. 10. Right anterior-oblique roentgenogram. Note the dilated right pulmonary artery.

FIG. 11. Left anterior-oblique roentgenogram. Note the clear retrocardiac space and the dilated left main pulmonary artery.

FIG. 12. Postero-anterior roentgenogram of patient of *Case 18*. Note the fine mottling of the lung parenchyma.

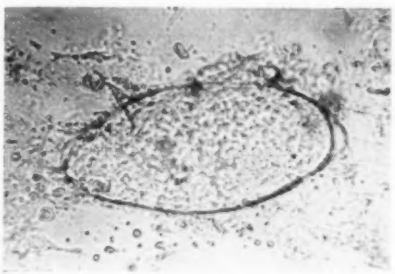

FIG. 13. Specimen of sputum of patient of *Case 12* showing ova of *Schistosoma mansoni*.

atrial septal defects (50). The downward thrust of the dilated right main pulmonary artery is very characteristic of schistosomal cor pulmonale. Severe exertional dyspnea with the absence of cyanosis also favors the diagnosis of chronic pulmonary schistosomiasis.

The disease has a prolonged course, and right ventricular failure may be very late in appearing (figure 14, table 3). In the late stages of the disease, avoidance of physical exertion is the main treatment. Patients with enormous aneurysmal dilatation of the pulmonary artery can be comfortably maintained in this way. Right ventricular failure is treated by customary bed rest, digitalis, and diuretics.

Pulmonary Function

The different clinical types of ventilatory insufficiency noted in 13 patients with schistosomal cor pulmonale are presented (table 4).

Various pulmonary function tests for intrapulmonary distribution of air and the diffusion of air through the alveolo-capillary membrane were also performed. Resting and exercise minute ventilations were both determined. The degree of dyspnea experienced by the patient during the performance of a standard exercise test was also estimated. Spirographic tracings of the total vital capacity and the maximal breathing capacity were recorded on a Collins respirometer. The timed vital capacity was measured on a standard Gaensler timed vitalometer (52). Residual volume was calculated

FIG. 14. Postero-anterior roentgenograms of patient of *Case 15*: (left) taken in July, 1955; (right) taken in March, 1957.

TABLE 3

HEMODYNAMIC DATA ON ONE PATIENT OVER A TWO-YEAR PERIOD

Case Number, Age, and Sex	State	Cardiac Index	Mean Pulmonary Arterial Pressure	Mean Pulmonary Capillary Pressure	Pulmonary Arteriolar Resistance (Arbitrary Units)
		1955			
		L/min./m²	*mm. Hg*	*mm. Hg*	
15 26 M	Rest	3.7	36	0	9.8
	Exercise	4.0	68	0	16.9
		1957			
	Rest	2.69	66	0	24.5
	Exercise	3.56	88	0	22.5

by the helium dilution closed-circuit method of Briscoe (53). The intrapulmonary distribution of air was evaluated as described by Briscoe (53). The mixing ratio was calculated in 10 cases of schistosomal cor pulmonale. The degree of arterial blood oxygen saturation at rest and during exercise was calculated. The impairment in diffusion was inferred from the unsaturation of the arterial blood after eliminating any possible defects due to ventilation and distribution (51, 54).

The pulmonary function values obtained in this study were compared with the normal values obtained from the predicted tables of Baldwin and associates (51). The nomenclature of Pappenheimer and associates was utilized throughout the text (55).

The maximal breathing capacity minus the respiratory minute volume for a given state of physical activity represents the breathing reserve. Cournand and co-workers (54) have demonstrated that the degree of severity of dyspnea can be evaluated by dividing the breathing reserve by the maximal breathing capacity and have termed this ratio the dyspneic index. When this index decreases to between 60 and 70 per cent, dyspnea is usually apparent. During the performance of the standard exercise test by the patients in the present study, the dyspneic index decreased to between 30 and 80 per cent, and moderate to severe dyspnea was noted in every case.

Three main clinical types of ventilatory insufficiency were demonstrated in the group of patients (figure 15). In 3 patients the pulmonary function tests were characteristic of obstructive ventilatory insufficiency (51, 56, 57). There was a decreased maximal breathing capacity, a normal total vital capacity, but a retarded timed vital capacity. The residual volume was increased and the ratio of residual volume to total lung capacity times 100 varied between 35 and 38 per cent. The spirographic tracing was characteristic of the obstructive pattern. Airway obstruction was the predominant defect in these patients.

In 3 other patients, pulmonary function tests demonstrated a restrictive type of ventilatory insufficiency (51, 56, 57), characterized by a reduction in the total lung capacity with a decreased total vital capacity and a normal timed vital capacity. The maximal breathing capacity was normal or only slightly decreased. The residual volume was normal, and the ratio of residual volume to total lung capacity times 100 was below 25 per cent. The spirographic tracing was typical of restrictive ventilatory insufficiency. In these patients loss of lung distensibility had occurred. No airway obstruction was demonstrated in the patients.

In 4 patients, combined obstructive and restrictive ventilatory insufficiency was demonstrated (56, 57), the pulmonary function tests being characterized by a decreased maximal breathing capacity and a reduction of the total vital capacity and timed vital capacity. The residual volume was slightly increased and the ratio of residual volume to total lung capacity times 100 varied between 26 and 58 per cent. Both airway obstruction and loss of lung distensibility had occurred in these patients.

In 2 patients the only pulmonary function defects were a delayed timed vital capacity. In one patient the pulmonary function tests were normal.

Briscoe (53) demonstrated in normal subjects that, when the rate of decrease in helium concentration is plotted logarithmically against the corresponding number of breaths, a straight line termed the mixing curve is obtained. In gross uneven ventilation of the lungs, an inflection of this straight line occurs. Mixing curves were plotted in 10 cases of schistosomal cor pulmonale, and in no case was there an inflection of the straight line (figure 16). Gross uneven ventilation of the lung, therefore, was not present in this group of patients.

At rest arterial blood oxygen unsaturation was present in only 2 patients, but on exercise an additional 3 patients demonstrated an arterial

TABLE 4

PULMONARY FUNCTION OF THIRTEEN SCHISTOSOMAL COR PULMONALE PATIENTS

Case Number, Age, Sex, BSA	TVC	Vital Capacity Per Cent Normal 1-sec. VC	3-sec. VC	MBC Per Cent Normal	MBC/VC= Air Velocity Index	Dyspneic Index		Total Lung Capacity Per Cent Normal	Ratio Residual TLC Per Cent	MR
						Resting BR/MBC	Exercise BR/MBC			
1 45M 1.66	86	50	88	56	0.65	80	48	102	36	0.36
2 50F 1.45	71	69	98	91	1.3	81	58	—	—	—
3 45M 1.76	61	50	94	46	0.75	79	—	—	—	—
4 34M 1.75	99.6	60	80	104	1.0	90	75	116	31	0.21
5 17M 1.48	66	80	94	92	1.4	86	79	67	13	0.33
8 28F 1.4	54	25	43	24	0.4	40	30	107	58	—
9 34M 1.61	91	53	76	88	0.97	87	66	98	38	0.35
10 30M 1.63	109	60	79	73	0.67	84	55	122	34	0.31
11 20M 1.61	77	81	96	87	1.1	83	43	85	22	0.28
12 40M 1.7	105	68	84	113	1.1	90	67	112	28	0.35
15 26M 1.48	86	68	83	85	0.99	85	62	89	26	0.37
16 50M 1.54	106	46	67	68	0.64	82	76	124	36	0.37
17 33M 1.62	96	81	93	122	1.3	90	70	108	31	0.30

Legend:
BSA = body surface area in square meters
TVC = total vital capacity
1-sec. VC = one-second vital capacity
3-sec. VC = three-second vital capacity
VC = vital capacity
MBC = maximal breathing capacity
BR = breathing reserve
TLC = total lung capacity
MR = mixing ratio

blood oxygen unsaturation (table 5). This blood gas disturbance could possibly be due to either physiologic shunting of the blood or to defects in alveolo-capillary diffusion.

Hemodynamics

Right heart cardiac catheterization was performed in the usual manner (58) on 13 of the 15 patients. In addition, 4 subjects with no evidence of cardiopulmonary disease were catheterized and served as a control group. Pulmonary capillary pressures were obtained by wedging the tip of the catheter in the peripheral lung field according to the method of Hellems and associates (59). Pulmonary arterial pressure and blood samples were taken through the catheter. An indwelling Cournand needle in the brachial or radial artery pro-

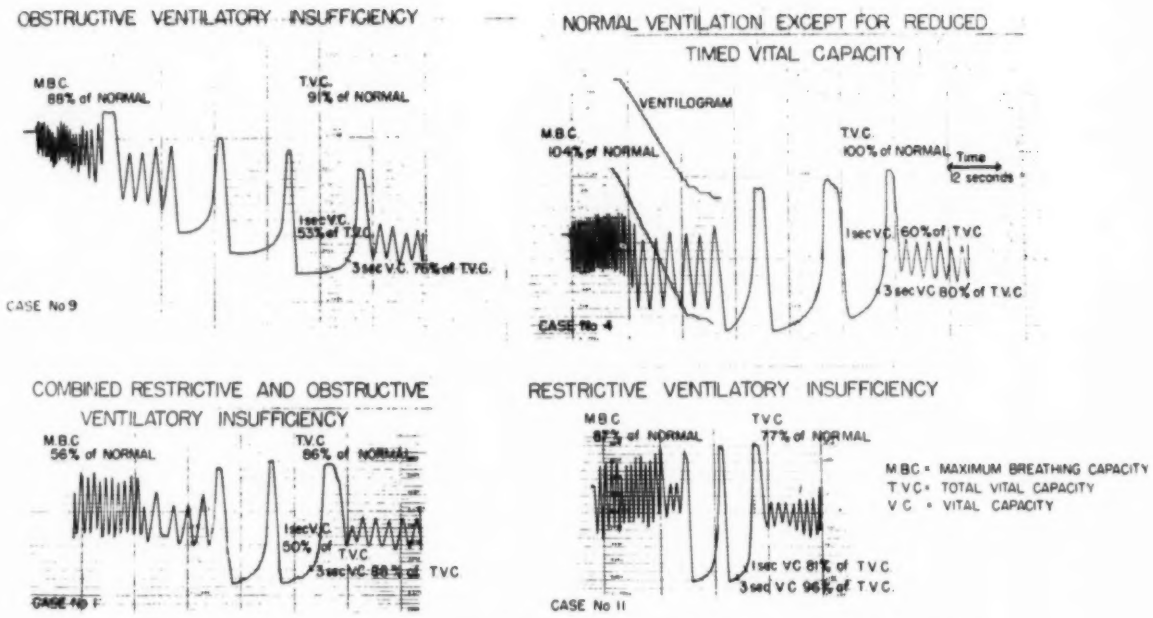
OBSTRUCTIVE VENTILATORY INSUFFICIENCY
MBC 88% of NORMAL
TVC 91% of NORMAL
1 sec VC 53% of TVC
3 sec V.C. 76% of TVC
CASE No 9
NORMAL VENTILATION EXCEPT FOR REDUCED TIMED VITAL CAPACITY
VENTILOGRAM
MBC 104% of NORMAL
TVC 100% of NORMAL
Time 12 seconds
1 sec VC 60% of TVC
3 sec VC 80% of TVC
CASE No 4
COMBINED RESTRICTIVE AND OBSTRUCTIVE VENTILATORY INSUFFICIENCY
MBC 56% of NORMAL
TVC 86% of NORMAL
1 sec VC 50% of TVC
3 sec VC 88% of TVC
CASE No 1
RESTRICTIVE VENTILATORY INSUFFICIENCY
MBC 87% of NORMAL
TVC 77% of NORMAL
1 sec VC 81% of TVC
3 sec VC 96% of TVC
CASE No 11
MBC = MAXIMUM BREATHING CAPACITY
TVC = TOTAL VITAL CAPACITY
VC = VITAL CAPACITY

FIG. 15. Pulmonary function (normal values from predicted tables of Baldwin and associates).

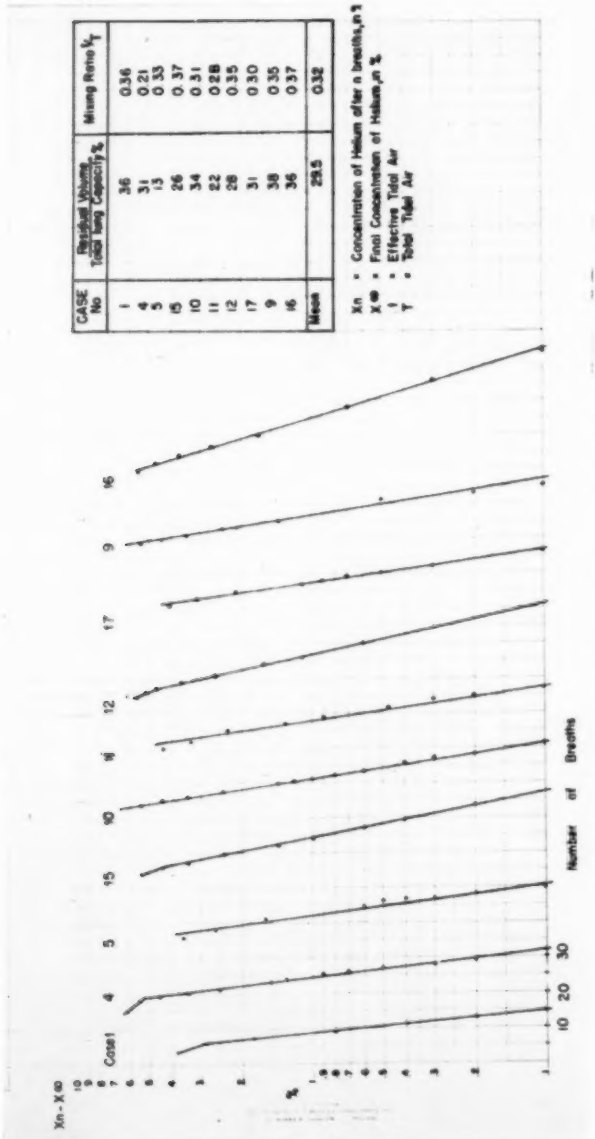

FIG. 16. Mixing curves of ten schistosomal cor pulmonale patients.

TABLE 5
PER CENT ARTERIAL BLOOD OXYGEN SATURATION

Case Number	Per Cent Oxygen Saturation	
	Rest	Exercise
1	93.5	90.8
2	94.8	93.9
3	96.1	91.0
4	94.8	95.3
5	96.8	96.6
8	90.6	84.8
9	96.1	94.2
10	97.8	98.0
11	97.2	94.8
12	95.1	94.8
15	87.7	85.0
16	94.6	92.6
18	95.3	95.3
19	94.5	—

TABLE 6
HEMODYNAMIC DATA IN FOUR NORMAL SUBJECTS

Case Number, Age, and Sex	State	Cardiac Index	Mean Pulmonary Arterial Pressure	Mean Pulmonary Capillary Pressure	Pulmonary Arteriolar Resistance (Arbitrary Units)*
		L./min./m²	*mm. Hg*	*mm. Hg*	
6	Rest	5.5	13	8	1.5
21M	Exercise	7.3	15	8	1.1
7	Rest	4.6	9	3	1.3
27M	Exercise	6.0	20	3	2.8
13	Rest	5.5	13	8	0.91
19M	Exercise	6.9	17	8	1.3
14	Rest	7.5	12	7	0.67
30M	Exercise	10.1	22	13	0.89
Mean values	Rest	5.8	12	6.5	1.1
	Exercise	7.6	18.5	8	1.52

* According to Goodale *et al.*, New England J. Med., 1955, *252*, 979.

vided systemic pressures and blood samples. Simultaneous pulmonary capillary pressures, pulmonary arterial pressures, systemic pressures, and electrocardiograms were performed on all patients. The pressures were measured through Sanborn manometers or through a Statham pressure transducer and were recorded on a Sanborn Cardiette Poly-Viso. Mean pressures were obtained electronically or by mechanical damping. In 3 patients oxygen consumption was determined on a McKesson metabolism apparatus. In the remaining patients the expired air was collected and the samples were analyzed in duplicate on a Haldane apparatus (60). Duplicate blood gas analyses were performed on the Van Slyke-Neill manometric apparatus (61). Cardiac outputs were calculated by the direct Fick procedure (62). Cardiac outputs, pulmonary pressure gradients, systemic pressures, and pulmonary and systemic vascular resistances were estimated at rest and during graded exercise performed on a bicycle ergometer.

In the 4 control subjects, the mean pulmonary arterial pressures, the mean pulmonary capillary pressures, the pulmonary arteriolar resistances, and the cardiac outputs were within normal limits (63, 64) (table 6). During the performance of exercise, an increase in the pulmonary blood flow occurred, accompanied by a minimal increase in the mean pulmonary arterial pressures and little change in the pulmonary arteriolar resistances (65–67).

In the 13 patients with schistosomal cor pul-

TABLE 7
HEMODYNAMIC DATA IN THIRTEEN SCHISTOSOMAL COR PULMONALE PATIENTS

Case Number, Age and Sex	State	Cardiac Index	Mean Pulmonary Arterial Pressure	Mean Pulmonary Capillary Pressure	Pulmonary Arteriolar Resistance (Arbitrary Units)
		L./min./m²	*mm. Hg*	*mm. Hg*	
1	Rest	2.7	65	7	21.3
45M	Exercise	3.3	88	6	24.6
2	Rest	2.2	63	12	23.4
50F	Exercise	2.5	71	10	24.1
3	Rest	3.1	49	6	13.8
42M	Exercise	3.4	93	6	25.9
4	Rest	2.8	64	4	21.7
34M	Exercise	4.0	100	4	24.2
5	Rest	5.8	45	6	6.8
17M	Exercise	6.1	63	6	9.4
8	Rest	4.4	37	7	7.3
28F	Exercise	4.8	58	15	9.2
9	Rest	5.0	41	12	5.9
34M	Exercise	6.2	70	18	8.4
10	Rest	2.6	66	10	21.9
35M	Exercise	2.9	78	10	23.2
11	Rest	2.3	67	7	26.4
20M	Exercise	2.6	78	8	26.9
12	Rest	2.5	50	10	16.0
35M	Exercise	3.7	73	13	16.0
15	Rest	3.7	36	0	9 8
26M	Exercise	4.0	68	0	16.9
16	Rest	2.1	20	3	8.0
50M	Exercise	2.6	31	3	11.0
18	Rest	3.3	53	0	16.0
35M	Exercise	4.2	66	0	16.0

monale, the mean pulmonary arterial pressures were elevated, the pulmonary arteriolar resistances were increased, and the pulmonary capillary pressures were normal. The pulmonary blood flow was reduced in 8 cases (table 7). On exercising, these patients showed a substantial elevation of the mean pulmonary arterial pressures, an increase in the pulmonary arteriolar resistances, and only a slight increase in the pulmonary blood flow. The pulmonary capillary pressures did not change. These observations indicate the restricted capacity of the pulmonary vascular bed (68) caused by the diffuse endarteritis obliterans.

Comment

Chronic pulmonary schistosomiasis is a disease mainly affecting the pulmonary vascular tree. The diffuse endarteritis obliterans greatly reduces the capacity of the pulmonary vascular bed, resulting in a raised pulmonary arterial pressure. Anoxia may play a secondary minor role in raising the pulmonary arterial pressure during exercise. The presence of an increased vasomotor tone contributing to the raised pulmonary arterial pressure has been suggested by De Faria (36) but has still to be proved.

Mild ventilatory insufficiency, whether obstructive, restrictive, or a combination of both, is present in the majority of patients with schistosomal cor pulmonale Gross uneven ventilation of the lungs does not occur and alveolo-capillary diffusion defects, if any, are slight.

Summary

The pathology of pulmonary schistosomiasis has been illustrated and the vascular nature of the disease stressed. The clinical picture and chest roentgenographic findings in 15 patients with chronic pulmonary schistosomiasis were presented. Pulmonary function tests performed on these patients showed evidence of mild ventilatory insufficiency, and right heart cardiac catheterization demonstrated an elevated pulmonary arterial pressure.

Sumario

Esquistosomiasis Pulmonar Crónica

Pónese de manifiesto la histopatología de la esquistosomiasis pulmonar, recalcándose la naturaleza vascular de la dolencia. Preséntanse el cuadro clínico y los hallazgos roentgenográficos en 15 enfermos que tenían esquistosomiasis pulmonar crónica. Las pruebas funcionales del pulmón ejecutadas revelaron signos de leve insuficiencia ventiladora y el cateterismo del corazón derecho mostró elevación de la presión arteriopulmonar.

Resume

Schistosomiase pulmonaire chronique.

La pathologie de la schistosomiase pulmonaire est illustrée et les auteurs mettent en évidence la nature vasculaire de la maladie. L'aspect clinique et les signes radiologiques décelés chez 15 patients atteints de schistosomiase pulmonaire chronique sont présentés. Les épreuves de la fonction pulmonaire effectuées chez ces malades ont révélé une insuffisance modérée de la ventilation et le cathétérisme du coeur droit a démontré une élévation de la pression artérielle pulmonaire.

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COEXISTING PRIMARY LUNG CARCINOMA AND PULMONARY TUBERCULOSIS[1]

A Report of Fifteen Cases Discovered Through a Chest Clinic and Hospital

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Introduction

Physicians concerned with diseases of the chest have been aware in recent years of an increase in the incidence of tuberculosis and carcinoma of the lung in older men. This trend in tuberculosis has been noted throughout the United States (1, 2) and is illustrated by changes which have occurred in hospital populations since 1940. For example, during that year, males forty-five years of age and older represented 5.9 per cent of the total admissions to Ray Brook State Tuberculosis Hospital. The corresponding figure for 1957 was 40 per cent, representing more than a sixfold increase. Primary bronchogenic carcinoma is at present one of the most frequently encountered forms of neoplasm in the male. Hilleboe (3) reports that in New York State the death rate for pulmonary carcinoma in men has increased by 570 per cent during the last two decades. At Ray Brook, lung cancer was rarely seen prior to 1940, but since that time a total of 137 cases have been recognized through the chest clinics and hospital. The present report concerns 15 of these cases in whom pulmonary tuberculosis coexisted with carcinoma of the lung. Two additional cases not included in this study were seen in which bronchogenic carcinoma and other granulomatous lung diseases occurred together. In one case, pulmonary histoplasmosis had been diagnosed for many years before the carcinoma was apparent, and in the other, acid-fast bacilli which were not tubercle bacilli were repeatedly cultured from the sputum.

The difficulties inherent in the early diagnosis of pulmonary carcinoma are well known and are increased when tuberculosis is also present. This retrospective analysis of experience with patients having the two diseases was undertaken in the hope that a review of the initial manifestations of pulmonary carcinoma might aid in earlier recognition. The importance of these cases is enhanced by the fact that in 5 instances serial roentgenographic observations for tuberculosis permitted visualization of the cancer from its early stage.

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

Observations

Analysis of Cases

The age of the 15 patients at the time of diagnosis of carcinoma varied between forty-two and seventy-two years, with an average age of fifty-eight. All were males. Of the 14 who were questioned concerning smoking, all had smoked since adolescence, and 5 had consumed more than twenty cigarettes daily for many years. The diagnosis of tuberculosis was confirmed by the recovery of tubercle bacilli from the sputum of 11 patients and by microscopic examination of surgical and necropsy tissue in 3. The remaining patient gave a history of treatment for active cavitary pulmonary tuberculosis for a period of twenty months in a United States Army hospital. He subsequently was followed in the clinics; his disease was inactive. The extent of the tuberculosis according to the *Diagnostic Standards* of the National Tuberculosis Association was minimal in 4 of the 15 patients, moderately advanced in 6, and far advanced in 4 patients. Neoplastic and tuberculous diseases were so intermixed in one case that the extent of the tuberculous disease could not be determined.

Roentgenographic Studies

Roentgenographic evidence of tuberculosis was found in the left upper lobe in 3, the right upper lobe in 3, both upper lobes in 2, and in all five lobes in 2 patients. The disease in the 5 remaining patients occurred in the following locations: both upper and left lower lobes, right upper and middle and left lower lobes, right lower lobe, left lower lobe, and indeterminate.

The site of origin of the primary carcinoma was ascertained in 14 of the 15 cases. The right upper lobe bronchus was involved in 8; the right lower

lobe in one; the left upper in one; the left lower in 3, and the left main bronchus in one patient.

A review of serial chest roentgenograms and of pathologic data was undertaken in order to determine whether there was correlation between place of origin of the tuberculosis and the carcinoma. In 7 patients, the carcinoma arose in an area where no evidence of previous tuberculosis was apparent. The two conditions occupied the same general area in the lung in 6 instances. In one case, carcinoma was diagnosed in a patient with a negative tuberculin reaction. Subsequently, the tuberculin reaction became positive and tubercle bacilli were found in the sputum. The primary site of origin of a diffuse alveolar cell carcinoma was not found in the remaining patient.

In 7 patients, the earliest roentgenographic evidence of primary carcinoma of the lung appeared as a circumscribed nodular homogeneous shadow in the middle lung zone in the region of the second or third bronchial divisions. Unilateral enlargement of the hilum was the presenting roentgenographic sign in 3 cases and was associated with relative airlessness of a segment of the lung in 2 of these. The first abnormality on the chest film in one patient was a spontaneous pneumothorax with an 80 per cent collapse of the lung. It could not be ascertained whether the pneumothorax was due to the carcinoma or the associated tuberculosis, both of which were found in the lung after re-expansion. Increased radiopacity of a portion of the lung due to relative airlessness without hilar enlargement was seen initially in 2 instances. In the remaining 2 cases, carcinoma was not recognized roentgenographically even in retrospect.

In most instances, the shadows in the chest film, which in retrospect were found to be due to carcinoma, increased in size rapidly over a period of several months. In one case, however, the change in size took place gradually over a number of years. These changes were entirely unrelated to the roentgenographic course of the tuberculosis. Not infrequently, dissimilar roentgenographic behavior of shadows in different parts of the lung was the clue which suggested the presence of a second disease. Pulmonary tuberculosis was the initial disease process in 10 of the cases. The time which elapsed between the diagnosis of tuberculosis and the earliest roentgenographic evidence of cancer varied between nine months and thirty-five years. It was more than four years in 9 of the 10 cases. In 4 cases, the diseases were recognized simultaneously. Carcinoma preceded the tuberculosis in the remaining patients.

The time interval between the first roentgenographic evidence of carcinoma and the recognition of the malignancy was between two and three years in 2 cases, about one year in 2 instances, and between eight months and one week in 9 patients. Carcinoma was diagnosed post mortem in the remaining 2 patients.

Diagnostic Methods

A review of the diagnostic methods employed reveals that cytologic examination of the sputum was positive for neoplasm in 2 of 9 patients, and of the bronchial aspirate in 5 of 7 patients in whom these tests were performed. Bronchoscopic appearance suggested malignancy in 6 of 9 patients who were bronchoscoped, and the bronchoscopic biopsy was diagnostic of carcinoma in 5 of 6 instances in which it was done. Pathologic examination of resected tissue confirmed the diagnosis of malignancy in 5 cases. In one additional patient, the tissue examined at biopsy was reported to show a chronic granuloma consistent with the diagnosis of tuberculosis. In several instances, more than one method was diagnostic in a single individual.

The pathologic classification of the 15 cases of neoplasm was as follows: epidermoid carcinoma, 5; anaplastic carcinoma, 5; alveolar cell carcinoma, 4; and unknown, 1.

Treatment and Results

In 9 cases, surgery was not performed. Five of the 6 patients who were surgically explored had pulmonary resection (one pneumonectomy and 4 lobectomies). In one patient who had a lobectomy, a contralateral segmental resection for tuberculosis had previously been done. Two of the 5 who had pulmonary resection are dead; 3 are alive without evidence of residual neoplastic disease. In these 3 patients, survival time following resection has been two years, one year, and five months, respectively. It is of interest that in these patients the time that elapsed between the first chest film in which cancer was found and resection was: three years and two months, one year, and nine months, respec-

tively. Among the 12 patients who died, survival time after the first roentgenographic evidence of carcinoma varied between two years and seven months, and three months. Three patients lived about two years and the remaining 7 less than nine months. In 2 patients, the neoplastic disease was not diagnosed during life.

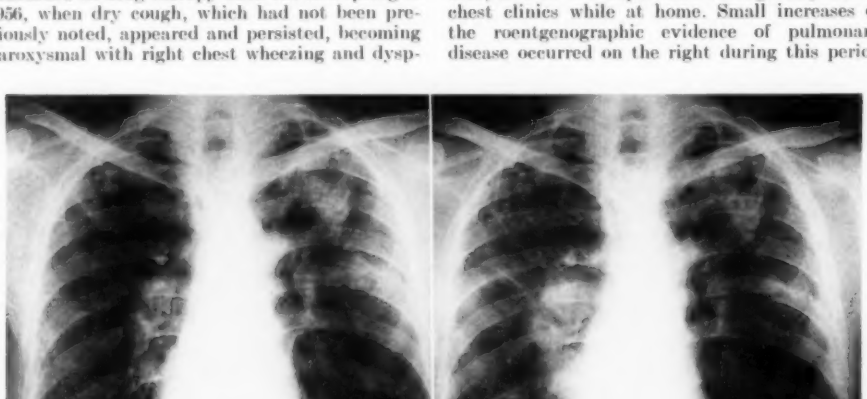
Some factors of importance in the early diagnosis of primary lung carcinoma in an individual with concomitant pulmonary tuberculosis are illustrated by the case histories of 5 patients in whom roentgenographic observations were available from the onset of the neoplastic disease.

Report of Cases

Case 1. M. Z., a 42-year-old male X-ray technician, had been treated intermittently at Ray Brook State Tuberculosis Hospital for slowly progressive pulmonary tuberculosis since 1942. Treatment included hospital care, right and left artificial pneumothorax and, during the third admission, drug treatment with streptomycin and PAS, totaling one year. Following discharge in May, 1953, he had been employed as an X-ray technician. The left artificial pneumothorax was continued. In February, 1955, pneumothorax refills were stopped and during the re-expansion period he was given alternate courses of streptomycin and PAS, and streptomycin and isoniazid. He continued on drug therapy until the late spring of 1956, when dry cough, which had not been previously noted, appeared and persisted, becoming paroxysmal with right chest wheezing and dyspnea. He was admitted to a sanatorium in June, 1956, where a chest film revealed that definite enlargement of the right hilum had appeared since the March, 1956, chest roentgenogram had been taken (figure 1). He was transferred to Ray Brook State Tuberculosis Hospital in August, 1956, when he was found to be acutely ill, dyspneic, and cyanotic. The hilar shadow had increased in size (figure 2). Acid-fast bacilli were found in the sputum, but cultures from this specimen did not grow. Bronchoscopy disclosed a firm yellow mass in the right main bronchus. The bronchial aspirate contained malignant cells, and a bronchial biopsy was reported as showing anaplastic carcinoma. Autopsy revealed, in addition to fibrocaseous tuberculosis of the lungs, an anaplastic carcinoma of the right main bronchus with metastases involving the mediastinum and periportal lymph nodes, liver, and pericardium.

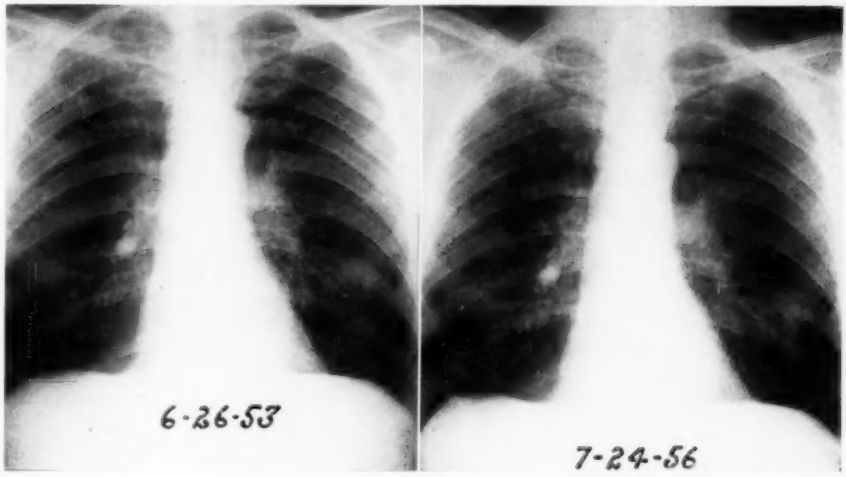
Comment: This case illustrates that an increase in symptoms and the appearance of unilateral hilar enlargement is of significance in a patient receiving adequate tuberculosis chemotherapy with sputum negative for tubercle bacilli.

Case 2. H. P., a 66-year-old male attendant, was employed in the tuberculosis ward of a mental hospital when a routine chest roentgenogram revealed pulmonary disease scattered over the upper half of the right lung. He was hospitalized at Ray Brook State Tuberculosis Hospital on five different occasions between September, 1949, and May, 1956, and he was supervised through Ray Brook chest clinics while at home. Small increases of the roentgenographic evidence of pulmonary disease occurred on the right during this period



Case 1

FIG. 1 (Left). Roentgenogram of chest. Note the right hilum.
FIG. 2 (Right). A rapid increase in the right hilar density has occurred over a period of three months.



Case 2

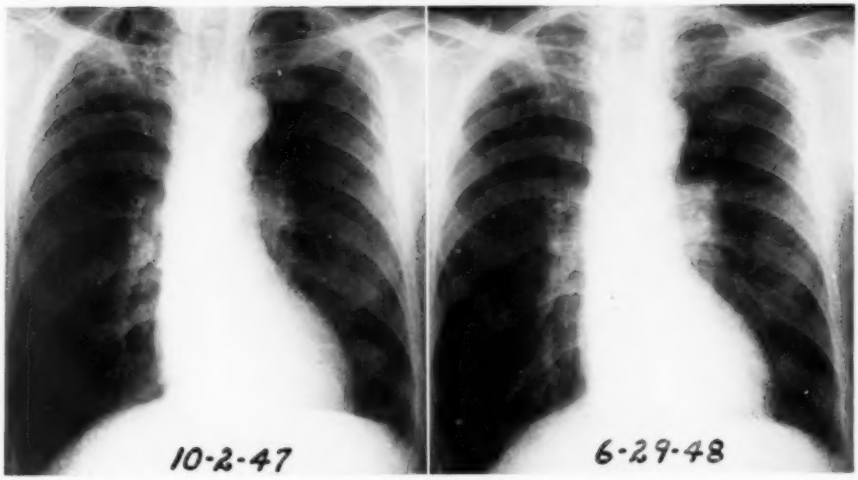
FIG. 3 (Left). In this roentgenogram an abnormal density is seen for the first time in the left lower lobe.

FIG. 4 (Right). Roentgenogram three years later. Note the slow increase in the size of the abnormal density in the lower left lung.

of time. On the left, at the time of the third admission, a chest film of June 26, 1953, disclosed for the first time an abnormal density in the lower lung (figure 3). Tubercle bacilli were repeatedly cultured from the sputum. The organisms were found to be susceptible to streptomycin, PAS, and isoniazid. Tripie-drug therapy was begun in June, 1955. Tubercle bacilli disappeared from the sputum in the second month of therapy, and the noninfectious state persisted. Because of a gradual increase in extent of the lesion in the left lower lobe, the patient was admitted to the sanatorium for the sixth time in May, 1956. The significant findings included physical and fluoroscopic evidence of moderate pulmonary emphysema. The chest roentgenogram showed no change on the right, but the area of disease in the left lower lung had increased in size (figure 4). Neither tubercle bacilli nor malignant cells were found in the sputum. Bronchoscopy revealed no abnormalities, but in the bronchial aspirate large cells with dark staining nuclei were seen. A resection of the left lower lobe was done on August 3, 1956, and an epidermoid carcinoma was found. The patient was discharged in November, 1956, and has remained well except for persistent dyspnea. No evidence of recurrence of the malignant disease was reported at the time of the last clinic examination in March, 1958.

Comment: The appearance of a discrete nodular density which increased in size in spite of effective drug therapy for tuberculosis in a patient without demonstrable tubercle bacilli in the sputum should alert the clinician to the possibility of a second disease.

Case 3. F. H., a 62-year-old male tuberculosis hospital employee, had always been well until a routine chest roentgenogram in May, 1943, revealed disease in the upper third of the right lung. A diagnosis of pulmonary tuberculosis was made. He was followed in the clinic without change in his condition and with noninfectious sputum until October, 1947. At that time, a homogeneous nodular density 1 cm. in diameter appeared in the chest film in the lower portion of the upper third of the left lung (figure 5). Thereafter, on serial chest roentgenograms the evidence of disease on the left slowly enlarged (figure 6) and eventually involved the left hilum. Cough and expectoration increased, hemoptysis occurred and, in January, 1950, a left pleural effusion appeared. On clinical examination on March 27, 1950, paralysis of the left leaf of the diaphragm and the left vocal cord was found. Bronchoscopic examination was refused. Malignant cells were not demonstrated in the sputum. On admission to Ray Brook State Tuberculosis Hospital on June 2, 1950, the patient was chronically ill, dyspneic at rest, and had a large left pleural effusion. Repeated thoracenteses temporarily relieved the dyspnea. Tubercle bacilli, malignant cells, or other microorganisms were not found in the pleural fluid. He died on November 7, 1950. At autopsy, an epidermoid bronchogenic carcinoma of the left lung with metastasis



Case 3

FIG. 5 (Left). Chest roentgenogram showing a faint abnormal density in the left first anterior interspace mesially.

FIG. 6 (Right). A definite increase in the size of the nodular density on the left has occurred.

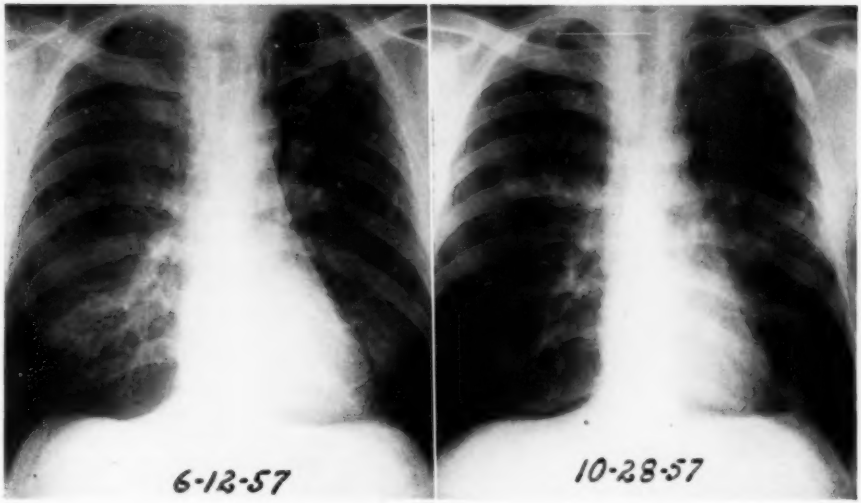
to the mediastinal lymph nodes, pleura, and right middle lobe, associated with fibrocaseous tuberculosis of the right upper lobe, was found.

Comment: In this patient, more than three years elapsed between the first roentgenographic evidence of cancer and death. Although the patient submitted many satisfactory specimens of sputum, tubercle bacilli were not found during the period when the disease progressed.

Case 4. C. H., a 61-year-old physician who was known to have had pulmonary tuberculosis since 1912, was discovered in 1943 to have an extension of disease in the right lung, with a cavity. He was treated with a five-rib right thoracoplasty that year, and two years later, in April, 1945, he was admitted to this hospital because of an increase of symptoms and the demonstration of tubercle bacilli in his sputum. Scattered bilateral pulmonary disease with right obstructive emphysema and markedly diminished pulmonary ventilatory function was found. He was discharged in June, 1946, with his disease classified as quiescent and thereafter he did light work until the fall of 1953, when because of increasing dyspnea he was readmitted on November 2, 1953. At that time, he was found to be markedly dyspneic and moderately cyanotic. No evidence of an increase of pulmonary disease was found on roentgenographic examination. Tubercle bacilli were not demonstrated in the sputum. Physical, roentgenographic, and electrocardiographic evidence of right heart failure was found and was treated with digitalis, diuretics, oxygen therapy, and phlebotomy. A severe emotional reaction to the situation failed to respond to sedative therapy, and a frank psychosis developed which necessitated transfer of the patient to the psychiatric division of a general hospital on December 3, 1953. He died three days later. The autopsy disclosed, in addition to bilateral pulmonary tuberculosis, a primary squamous cell carcinoma of a branch of the left lower lobe bronchus, with metastasis to the adjacent hilar lymph nodes.

Comment: Carcinoma in this case was not suspected during life. Roentgenographically, even in retrospect, it was not visualized in the films taken. To what extent the carcinoma contributed to cardiorespiratory death in this case is not clear.

Case 5. C. K. was a 50-year-old male cook in whom bilateral pulmonary tuberculosis involving the upper third of both lungs with cavity on the left was diagnosed in February, 1956. He was treated in a sanatorium with streptomycin and isoniazid between February, 1956, and October 8, 1956, when a resection of the apical posterior segment of the left upper lobe was performed. Drug therapy was continued until discharge on April 16, 1957. Isoniazid alone was recommended as home therapy but he did not take the drug. He returned to the sanatorium in August, 1957, because of left chest pain but left against advice



Case 5

FIG. 7 (Left). A small nodular density is noted for the first time in the right third anterior interspace. It was not apparent in the March or April, 1957, chest roentgenograms.

FIG. 8 (Right). Four months later. Note the definite increase in the size of the nodule on the right.

after one day in the hospital. He was referred to Ray Brook State Tuberculosis Hospital in October, 1957, when he was found to be acutely ill with a left pneumococcal pneumonia which cleared rapidly on penicillin therapy. On the right side, a nodular density 1.5 cm. in diameter was noted in the upper half (figure 8). A retrospective review of former films revealed that this shadow was barely discernible in a June 12, 1957, film (figure 7) but was not apparent in the March or April, 1957, chest roentgenograms. It was about 5 mm. in diameter when first seen and had increased gradually in size during the year prior to the Ray Brook Hospital admission. Neither tubercle bacilli nor malignant cells were found in the sputum. Bronchoscopy and scalene node biopsy did not reveal evidence of carcinoma. A right upper lobectomy was done on December 2, 1957. The surgical specimen contained an alveolar cell carcinoma. The patient was discharged to his home in December, 1957, in good condition, and has remained well.

Comment: The appearance of nodular shadows in the chest film of a patient must be viewed with suspicion when the sputum contains no tubercle bacilli and the patient has had satisfactory surgical and drug therapy for tuberculosis.

DISCUSSION

From the above experience and that of others (4–7), it is clear that the diagnosis of concomitant carcinoma in a case of pulmonary tuberculosis is aided through the recognition by the physician of a clinical course which differs from that usually encountered in uncomplicated pulmonary tuberculosis. Such factors as an increase of symptoms and/or progressive roentgenographic evidence of disease, in a patient with sputum free from tubercle bacilli and under effective drug therapy for tuberculosis, should prompt the clinician to search for more than one disease. Clearing of roentgenographic evidence of disease under drug therapy in one area with an increase of the shadows in another portion of the lung is of significance. A persistent expiratory rhonchus has been described in the era before antituberculous drug therapy as a sign of endobronchial tuberculosis (8). Current drug therapy has proved so effective in the treatment of tuberculous endobronchitis that the development of a persistent rhonchus should alert the clinician to the possibility of endobronchial tumor.

If the diagnosis of neoplastic disease in these cases is to be effective from the point of view of therapy, it must be recognized in most instances before symptoms or physical signs are present. It is of interest that in the present series the 3 patients who had pulmonary resection and are to

date without demonstrable metastasis had no symptoms prior to surgery. Two of these cases were not proved cytologically before surgical exploration. Thus, in this series, the earliest evidence of a concomitant carcinoma in the presence of pulmonary tuberculosis was found in the chest roentgenogram. The features which favor the diagnosis of carcinoma are as follows:

The development of a discrete nodular density in the lung, especially if it occurs in the lower lobe or the anterior portion of the upper lung. The earliest shadow might readily be mistaken for a spread of tuberculosis, but the shadow due to carcinoma is usually homogeneous rather than mottled and occurs in the mid zone rather than in the peripheral portion of the lung. A slow or rapid increase in size usually occurs and this may be unrelated to the clinical course of the tuberculosis.

Unilateral enlargement of the hilum with or without peritracheal lymph node adenopathy.

Relative airlessness of a segment, lobe, or the entire lung, or the appearance of the homogeneous density involving a segment or larger area of the lung.

Pulmonary neoplasm must be considered in the differential diagnosis of spontaneous pneumothorax. This fact will favor frequent and careful observation of such cases during the re-expansion period with suitable investigation when the re-expansion is delayed or incomplete. No characteristic roentgenographic features were noted even in retrospect in 2 cases.

Similar roentgenographic findings which suggest a carcinomatous element in cases of pulmonary tuberculosis have been described by others (4, 5, 9, 13), notably by Hauser and Glazer (9), who record as characteristic "a nodular density greater than 3 cm. in diameter especially if it is in the lower lung field." In 3 cases from the present series the earliest abnormal roentgenographic shadow which subsequently was found to be due to pulmonary carcinoma was a nodule which was considerably smaller than 3 cm. in diameter. It is believed that small nodular densities, especially if they appear spontaneously during treatment in a case of pulmonary tuberculosis, must be viewed with suspicion.

In this series, the right lung was involved in 9 and the left in 5 instances. In one case, bilateral involvement with an alveolar cell carcinoma was found. The preponderance of cases in which the primary carcinoma developed on the right is in accord with the observations of Bender (5).

It has been suggested that primary pulmonary carcinoma may arise at the site of previous tuberculous or bronchiectatic disease, i.e., in scar tissue or lung calcifications (10–12). In the present series, this connection was found in less than half of the patients in whom the original site of the carcinoma was determined.

In 10 of the cases, carcinoma appeared late in the course of the pulmonary tuberculosis at a time when the disease was stable or inactive. New disease, when it is found in such cases, is assumed to be the result of reactivation of tuberculosis. The difficulties in the recognition of a second disease entity are illustrated by the fact that in 4 of the 10 patients a period of one to three years elapsed between the discovery in the chest roentgenogram of a shadow, which in retrospect proved to be carcinoma, and its correct identification. The failure to demonstrate tubercle bacilli in the sputum in the presence of symptoms and increasing disease was found to be significant. Conversely, the demonstration of tubercle bacilli in the sputum does not exclude the presence of carcinoma when other features of the case, both clinical and roentgenographic, suggest the possibility of neoplastic disease.

The diagnostic methods employed in primary pulmonary carcinoma are well known. Simultaneous use of multiple diagnostic studies is advisable. In addition to history, the physical examination, and roentgenographic studies (including fluoroscopy and planigraphy), the writers routinely have a series of at least three consecutive sputum specimens examined for malignant cells. Sputum is collected directly into Zenker's solution. Bronchoscopy and biopsy of either cervical lymph nodes or scalene fat pad are scheduled together. Biopsy, via the bronchoscope, is done if an endobronchial lesion is found. In all cases, bronchial washings are collected for examination for cancer cells. Time-consuming repetition of diagnostic procedures is not advisable. Early thoracotomy with examination of a lung biopsy by frozen section to guide the surgeon is favored. It is believed that the risk of thoracotomy done with antituberculous drug therapy is relatively small and is justified when concomitant carcinoma is suspected. It is only through early exploration that the cases in which a small relatively slow-growing carcinoma amenable to surgical removal may be recognized and successfully treated.

It is noteworthy that in the small series of 15 cases, 4 patients with alveolar cell carcinoma were found. In the writers' experience, an apparent recent increase has occurred in the number of alveolar cell carcinomas which are recognized at this hospital.

Summary

A series of 15 patients with coexisting primary lung carcinoma and pulmonary tuberculosis has been analyzed and the case histories of 5 patients in whom the onset of the carcinoma was observed clinically and roentgenographically are presented in detail. Carcinoma was found to develop in an area where previous tuberculosis had existed in less than half of the cases. Certain variations from the usual clinical and roentgenographic course of pulmonary tuberculosis that suggest the presence of carcinoma are described. On the basis of the study of the 15 cases, prompt simultaneous utilization of all available diagnostic methods for the detection of primary lung carcinoma in a case of pulmonary tuberculosis is urged and early exploration is recommended.

Sumario

Coexistencia de Carcinoma Pulmonar Primario y Tuberculosis Pulmonar

Se analiza una serie de 15 enfermos en quienes coexistían carcinoma pulmonar primario y tuberculosis pulmonar, pormenorizándose las historias clínicas de 5 pacientes en quienes se observó clínica y radiográficamente la iniciación del carcinoma. Según se notó, el carcinoma se desarrolló en menos de la mitad de los casos en una zona en que había existido tuberculosis antes. Describense ciertas discrepancias de la habitual evolución clínica y radiográfica que sugieren la existencia de carcinoma. A base del estudio de los 15 casos, se encarece la rápida y simultánea utilización de todos los medios disponibles para el diagnóstico del carcinoma primario del pulmón en un caso de tuberculosis pulmonar y se recomienda la exploración temprana.

Resume

Cancer primaire du poumon avec tuberculose pulmonaire coexistante

Analyse des observations d'un groupe de 15 malades atteints de cancer primaire du poumon avec tuberculose pulmonaire coexistante; les antécédents médicaux de 5 malades chez lesquels le début du cancer avait été observé radiologiquement sont présentés en détail. Le cancer s'était développe dans moins de la moitié des cas en un point où une lésion tuberculeuse avait existé auparavant. Les auteurs décrivent certaines variations de l'évolution clinique et radiologique habituelle de la tuberculose pulmonaire suggérant l'existence du cancer. En se fondant sur l'étude de 15 cas, l'utilisation simultanée prompte de toutes les méthodes de diagnostic disponibles pour la détection du cancer pulmonaire primaire est instamment recommandée dans un cas de tuberculose pulmonaire ainsi qu'une exploration précoce.

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RESPIRATORY FUNCTION IN TUBERCULOSIS AND OTHER CHRONIC DISEASES OF THE LUNG[1, 2]

Translation of a Soviet Review

E. S. STEPANYAN

(Translation received for publication October 6, 1958)

Introduction

The interest of chest physicians in the study of respiratory function has grown, particularly in connection with the wide introduction of surgical methods of treatment. In the majority of clinics at the present time, the problems connected with the indications for surgical intervention and postoperative prognosis are decided on the basis of a careful functional study of the respiratory and the cardiovascular systems.

The contemporary methods of study—fractional bronchospirography (performed at rest and with exercise), oxyhemometry, catheterization of the pulmonary artery and of the heart, and also the use of labeled atoms—have greatly enriched the ideas of the mechanism of damage to respiratory function in tuberculosis.

Characteristics of the degree of disruption of respiration

At the present time, efforts continue to be made to determine and to express concretely the degree of disturbance of respiration in tuberculosis in connection with the character and severity of the disease. Hisato Anno and Tomashefski (1), studying the external respiration and the blood gases in 25 patients with tuberculosis of the lungs without any impairment of the function of the cardiovascular system, observed the following:

In limited tuberculosis of the lungs, disturbance of ventilation or changes of lung volume are not noticeable. In cases of damage of a segment or of a large part of a lobe of the lung, a certain increase in the admixture of venous blood can be noticed in the pulmonary capillaries. With moderately expressed and far advanced pulmonary tuberculosis, when the destruction is particularly marked, the disturbance of respiratory function is especially clearly defined. This disturbance is characterized by a lowering of the maximal volume of respiration and an increase in the admixture of venous blood in the pulmonary capillaries to more than 38 per cent. The same thing can be observed in patients with pulmonary emphysema.

Starting with the above, the writers believe that the disturbance of the interrelations between the blood circulation and the gas exchange in the lungs is connected with an increase in the admixture of venous blood in the pulmonary capillaries. In the far advanced forms of pulmonary tuberculosis, there is an increase in the ratio of residual air to the general lung volume, with a low diffusion of oxygen into the blood. In some of the patients, the reduction of diffusion of gases in the lungs is related to the decline in ventilation of the dead space. In patients with tuberculosis of the paratracheal lymph nodes, an increase is also noted in the admixture of venous blood in the pulmonary capillaries, especially in the severe course of the disease; the writers relate this to the obturation of the bronchus.

Rossier (2), on the basis of results of spirometric studies, analyses of the blood gases, and bronchospirometric studies in disease of the lungs, distinguishes three types of respiratory insufficiency: (*1*) hidden respiratory insufficiency with increase of the pulmonary ventilation and normal oxygen saturation of the blood; (*2*) partial insufficiency (the most frequently encountered type) with increased ventilation, sometimes a decline in the arterial oxygen saturation, but with a normal carbon dioxide content; (*3*) complete respiratory insufficiency with profound disturbance of oxygen and carbon dioxide transport, accompanied by a lowering of the alveolar ventilation, and, in consequence of this, an insufficient uptake of oxygen and removal of carbon dioxide.

Comparing the clinical picture of the disease with the oxygen pressure in the arterial blood,

[1] From Contemporary Problems in Tuberculosis, 1956, 7(4; 40): 3–13.

[2] Translation of this paper has been furnished by the National Institutes of Health, Public Health Service, as a part of their Russian Scientific Translation Program.

Lindenschmidt (3) divides the patients with disseminated tuberculosis or with bronchiectatic changes in the lungs into three groups: patients with hypoxemia of the first degree (pressure of oxygen in the blood, 87 to 85 mm. of mercury); patients with hypoxemia of the second degree (pressure of oxygen in the blood, 74 to 60 mm. of mercury); patients with hypoxemia of the third degree (pressure of oxygen in the blood, less than 60 mm. of mercury).

Bühlmann and associates (4), on the basis of a study of external respiration, blood gases, and electrocardiographic and oxyhemometric data, subdivides patients according to the character and degree of cardiopulmonary insufficiency into four groups: patients with patent ductus arteriosus and with arteriovenous aneurysms; patients with stenosis of the mitral valve and congestive manifestations in the pulmonary circulation; patients with pulmonary sclerosis; and patients with pulmonary emphysema.

Prior to a major operation on the lungs, it is essential to calculate not only the apparent pulmonary insufficiency at rest, but also that which appears under physical stress. With this, there arises a very complicated task, that of distinguishing pulmonary from cardiac insufficiency. With this objective, Valentin and Venrath (5) proposed to study the ventilation and the gas exchange at the time of physical stress (60 and 120 volts) with ordinary respiration and with respiration in an atmosphere of oxygen. The writers indicated that, in the patients with pulmonary insufficiency, the oxygen deficit arising with ordinary respiration could be removed upon transfer into an atmosphere of oxygen; in the presence of cardiac insufficiency, this deficit remains.

On the basis of a study of 500 miners suffering with chronic disease of the lungs, Motley (6) introduced the concept of the "ventilatory factor," which is computed from the following values: the 3-second vital capacity of the lungs, the limit of ventilation, and the amount of residual air.

For the determination of concealed respiratory insufficiency in patients with pulmonary tuberculosis and silicosis, Pasargiklian and his associates (7) used the method of Margaria, according to which the magnitude of energy expenditure is determined under increasing physical stress on the bicycle ergometer. The expended energy is expressed in this test by the ratio of energy metabolism to pulmonary ventilation (calories per liter). The writers established that a premature decline of this ratio is a sign of respiratory insufficiency. In patients with severe lung damage the value of this ratio is low even at rest, and with minor effort it continues to decline sharply.

For differentiation of insufficiency of the left heart with congestion in the pulmonary circulation from insufficiency of the right heart, Knipping (8) recommends measurement of the blood pressure directly in the pulmonary artery (by means of catheterization), the use of labeled atoms, study of the blood gases, and spirographic studies at rest and with effort.

The abundance of diverse methods offered for the determination of the degree of respiratory insufficiency, and for the differentiation of it from cardiac insufficiency, indicates the presence of great difficulties in the solution of these problems.

Respiratory Function in Artificial Pneumothorax and Artificial Pneumoperitoneum

The use of pneumothorax and pneumoperitoneum at the present time has been curtailed in connection with the use of antimicrobial therapy. Abroad, especially in the United States, a negative attitude has developed toward artificial pneumothorax, without sufficient basis for such an attitude. Certainly, following the imposition of pneumothorax a decline occurs in the functional indices of respiration. The respiratory function of the collapsed lung is reduced with the prolonged use of pneumothorax, especially when it is complicated by pneumopleuritis. But it must not be forgotten that the antibacterial preparations have sharply reduced both the prolonged use of pneumothorax and the possibility of its being complicated by pneumopleuritis.

Bolt and associates (9) indicate a lowering of ventilation and of oxygen consumption on the side of the pneumothorax (in investigations with the bronchospirographic method).

Bucher and Gloor (10) conducted bronchospirographic investigations on 21 patients following discontinuation of pneumothorax for one to seven years, without clinical signs of respiratory insufficiency. Eleven patients, in whom the pneumothorax was not complicated by pneumopleuritis, after discontinuation of the pneumothorax, showed no evidence of disturbance

in respiratory function; on the side of the pneumothorax, the minute volume of respiration was 80–97 per cent of the ordinary level, the vital capacity was 78 per cent; the oxygen consumption was 85 per cent. In 10 patients in whom obliteration of the pleural cavity developed, a substantial lowering of the respiratory indices was observed: on the side of the pneumothorax the minute volume constituted 50 per cent of the normal; the vital capacity, 47 per cent; and the oxygen consumption, 45 per cent of the normal amount.

Pätiälä and Karnoven (11) studied respiratory functions in 47 patients with tuberculosis one to three years after the termination of pneumothorax. The closed-type of Benedict-Roth apparatus was used. In the patients studied there were no active process, fibrotic changes in the lungs, or clinical signs of respiratory insufficiency. The mobility of the diaphragm was determined roentgenographically. According to the character of the changes in the pleura and diaphragm, the patients were divided into three groups. The first group comprised patients with uni- and bilateral pneumothorax, in whom the changes in the pleura and diaphragm were insignificant. Thickening of the pleura was found at the level of the lower lung field; the diaphragm either completely preserved its mobility or its movements were slightly limited by adhesions. The second group comprised patients with unilateral pneumothorax in whom the changes were moderately expressed; thickening of the pleura was disseminated and up to the middle lung field; the mobility of the diaphragm was sharply limited. In the third group were placed patients with uni- and bilateral pneumothorax in whom a number of marked changes were noted: the pleura was markedly thickened over all surfaces up as far as the apices; the diaphragm was completely stationary and immobile.

In patients of the first group the respiratory function was at the limits of normal. In patients of the second group a moderate reduction was observed in maximal ventilation and vital capacity, but among them were also encountered patients with normal functional indices. The writers attribute this to individual anatomic peculiarities of the disease and to the training of the patient. In the patients of the third group were observed clear-cut lowering of the vital capacity and of its fractions, of the maximal ventilation, and an increase in the volume of residual air.

Thus, the writers succeeded in establishing a connection between the disruption of respiratory function and the changes of the pleura and the limitation of motion of the diaphragm. In the absence of these complications the respiratory function, after the conclusion of pneumothorax, changed little.

Hertz (12) remarks that, following pneumothorax, hypoxia is encountered more frequently in patients with pleural adhesions than in patients with extensive pleural adhesions following exudative pleuritis (the mean per cent of arterial oxygen saturation following exudative pleuritis is 92.9, and after pneumothorax, 91.6).

Bruce (13) indicates a significant limitation of mobility of the lungs and a reduction of ventilation and of gas exchange following pneumothorax. Proceeding from this, the writer concludes that the indications for the imposition of artificial pneumothorax must be studied with the same care as the indications for a major operation; the formation of adhesions following pneumothorax sharply compromises the respiratory function. It is not necessary to maintain the pneumothorax for a long time after the appearance of exudate and thickening of the pleura, but at the moment of appearance of pneumopleuritis it is essential to undertake a whole complex of measures with the object of preventing the formation of adhesions. It is recommended that the patient lie on his abdomen or his healthy side and that he use therapeutic exercises; some recommend even the use of stimulation of the diaphragm with an electric current.

Siebens and associates (14) state that, in the absence of pneumothorax, pneumoperitoneum exerts a favorable influence on respiration. Judging by the roentgenographic findings, after pneumoperitoneum the respiratory movement of the diaphragm and the ribs not only is not limited but even is enhanced. The writers believe that after pneumoperitoneum the conditions of gas exchange in the lungs are improved due to the diminution of the volume of residual air and to the lowering of the contents of nitrogen in the lungs. The volume of residual air in patients was reduced, on the average, by 20.4 per cent, and the vital capacity by 4.4 per cent. In contrast to the patients, in 10 healthy persons studied after

pneumoperitoneum, the volume of residual air declined by only 2.8 per cent.

Respiratory Function in Patients with Pulmonary Tuberculosis upon Surgical Intervention

The influence on respiration of avulsion of the phrenic nerve: The majority of writers indicate that, despite the seeming lack of trauma in the operation of phrenico-exeresis and phrenico-alcoholization, these procedures exercise a very basic influence on the respiratory function of the tuberculous patient.

Bolt and associates (9), using the bronchospirographic method, showed that avulsion of the phrenic nerve on the side of operation elicits a significant fall in pulmonary ventilation and gas exchange. Rossier (2), using the methods of bronchospirography, spirography, and also analysis of the gases of the blood, showed that the disturbance of the respiratory function following phrenico-exeresis is expressed even more sharply than when following thoracoplasty.

Heine and Hell (15), on the basis of studies of 110 patients before and after phrenico-exeresis, established that the functional disturbances of respiration after the operation were comparatively insignificant if the phrenico-exeresis was preceded by the use of pneumothorax on the same side, but were sharply expressed in the presence of pleuro-diaphragmatic adhesions on the side of the operation. The decline in vital capacity and maximal ventilation was expressed the more strongly, the greater the mobility of the diaphragm prior to the operation. Within ten days following phrenico-exeresis, a diminution of the vital capacity on the average of 18.5 per cent was observed, chiefly due to the reduction of the volume of supplementary air by 24.4 per cent (the volume of reserve air fell by 14.4 per cent). The minute volume of respiration declined by 2.4 per cent, and the maximal ventilation by 10 per cent. Repeated studies on 40 patients fifty days after operation revealed a return of the value of the vital capacity and of ventilation to the original level and even higher. On the average, the vital capacity in these patients and the fractions of the vital capacity increased by 2 per cent, the minute volume by 2 per cent, and the value of the maximal ventilation by 8 per cent. Hence the data of the investigation of Heine and Hell allow us to believe that the lowering of the vital capacity and of pulmonary ventilation following phrenico-exeresis in patients with pulmonary tuberculosis bears a clear-cut but reversible character.

The influence of thoracoplasty on respiration: In a number of cases, thoracoplasty, exerting a positive therapeutic effect on the fundamental process, later leads to the development of cardiopulmonary insufficiency.

Considering this, in stating the indications for thoracoplasty, Thomas and Steele (16) employ the magnitude of the ratio of the ventilation upon walking to the maximal volume of respiration; thoracoplasty is recommended when the ratio is 0.3 or lower. A ratio of 0.5 indicates the presence of respiratory insufficiency. The determination of this value was performed on 129 patients. The operation was not performed on 16 patients, since the value of the ratio was higher than 0.35. In 113 patients it was 0.3 and lower. In not one of the latter cases were there any complications in the postoperative period.

Fleischer-Mölin (17) points to the significant drop in the arterial oxygen saturation after the thoracoplasty. He explains this by the admixture of insufficiently aerated blood to the arterial blood from collapsed portions of the lung, where circulation has been maintained but where there is poor ventilation. With hemoxytensiometer studies, it turned out that the oxygen pressure in the arterial blood in thoracoplasty is reduced by 15 to 40 mm. of mercury, while the decline with pneumothorax is 5 to 25 mm. of mercury. The degree of decline of oxygen pressure in the arterial blood after thoracoplasty does not correspond to the degree of diminution of the vital capacity of the lungs.

Hence, these data, to a considerable degree, explain the mechanism of the development of cardiopulmonary insufficiency after thoracoplasty and indicate the paths by which this complication may be anticipated, using the elemental studies of pulmonary ventilation (16).

The influence of pneumonectomy, lobectomy, and segmental resection of the lung on respiration: The study of pulmonary ventilation and of gas exchange after the removal of the lung or of a part of it has made it possible to clarify a number of complicated problems of the pathophysiology of respiration. The removal of the lung, lobe, or segment in the majority of patients is accompanied by a diminution in ventilation and gas exchange. In a number of cases after pneumo-

nectomy, lobectomy, or segmental resection, the ventilatory and gas exchange indices rose. Such a result is frequently noted in patients with removal of the lower lobes when, along with the lobectomy, there is a freeing from massive adhesions of the lower ribs and diaphragm. It is also noted upon the removal of severely damaged portions of the pulmonary tissue, not infrequently with marked changes of the bronchi leading into them. Effects of the operation which are positive in the functional sense cannot be explained only by the removal of the mechanical causes of the disturbances of normal ventilation and of blood circulation in the lungs, or by the removal of a weakly functioning lung, as the majority of writers tend to do. The very fact of removal of the basic pathologic focus of massive caseation undoubtedly plays a role also.

Hisato Anno and Tomashefski (1) indicate the elevation of the arterial oxygen saturation, the fall in the percentage of admixture of venous blood in the capillaries, and the decline in the ventilation of dead space after pneumonectomy. The writers relate this to the removal of a weakly functioning lung.

Vargha and Baktai (18), on the basis of investigations of 200 patients before lung resection and of 100 of them at various dates following operation, remark on the decline of vital capacity by 13 per cent and of maximal ventilation by 10 per cent after pneumonectomy. Lobectomy reduces the vital capacity by 7 per cent, not changing the maximal ventilation. Segmental resection reduces the vital capacity by 8 per cent and the maximal ventilation by 1 per cent. In the presence of complications (empyema, paralysis of the diaphragm), these figures are correspondingly increased. On the contrary, with a well-performed operation, when a part of the lung is removed (the function of which was sharply impaired), an improvement is observed in respiratory function which is especially noticeable with resection of the lower lobes of the lung. The present writer explains this by the circumstance that the lower ribs and the diaphragm are freed of adhesions. The improvement in the respiratory function after operation is explained also by the removal of the obturation of the corresponding branch of the pulmonary artery and bronchi draining the resected portions of the lung, and also the heightening of the effectiveness of ventilation in the alveoli in the postoperative period.

Warren and associates (19), on the basis of comparisons of bronchospirometric and gas analysis data in 74 patients with pulmonary tuberculosis and following removal of the lung and its components, came to the following conclusions:

The averages of the indices of maximal ventilation and of vital capacity of the lungs before operation show little correspondence with the extent of the disease process.

The averages of the indices of oxygen consumption show a correspondence with the extent of the disease process.

Following the resection of seven to ten segments, the maximal ventilation, on the average, declines by 13 per cent; and the vital capacity, by 18 to 22 per cent.

Additional applications of thoracoplasty to fill in the remaining cavity do not exercise a noticeable influence on the indices of ventilation, but are substantially reflected in the bronchospirometric indices, especially in the oxygen consumption.

Limburg (20) gives data of a bronchospirographic study of 87 patients with pulmonary tuberculosis prior to operation and within six months after pneumonectomy, with an uncomplicated postoperative course. In this, the writer uses not absolute but relative values (in per cent). On the basis of his findings the present writer affirms the following:

After resection of a lung, the vital capacity diminishes less sharply than the oxygen use.

Diminution of respiratory function after resection is greater than might be expected. Thus, resection of the upper lobe of the right lung leads to a reduction of respiratory function by 45 per cent in comparison with the normal function of the right lung, although, judging by the number of segments resected, this reduction ought to be only 30 per cent. After the removal of the superior and posterior segments of the right upper lobe, the reduction of respiratory function is 30 per cent, although, by calculation, it ought not to exceed 20 per cent. From this the necessity develops of a serious study of respiratory function in patients prior to operation and of the maximal sparing of healthy lung tissue.

Overholt and associates (21), on the basis of a detailed study of the external respiration before and after physical effort, and also of fractional bronchospirography, indicate the absence of disturbance of the respiratory function after segmental resection of the lung. The operations were performed in connection with bronchiectatic disease, elicited in one case by tuberculosis, and in

another by a lung abscess. The writers explain this by the compensatory function of the healthy lung tissue.

Inconsistent findings of various writers on the influence of the removal of the lung and its components on respiratory function are explained by the dissimilar composition of the groups of patients studied. The respiratory function of a patient in the postoperative period is determined not only by the amount of lung tissue removed, but also by the character of the changes both in the resected and in the preserved portions of the lungs, and also by the status of the cardiovascular and the nervous systems. In order to avoid the development of cardiopulmonary insufficiency in the postoperative period, it is necessary to test the functional potentialities of the respiratory and cardiovascular systems prior to the operation.

Explanation of certain mechanisms of impairment of respiratory function in tuberculous patients. The influence of pleural adhesions on respiratory function: A number of writers have attempted to study the influence of pleural adhesions on the respiratory function outside of phases of exacerbation of the tuberculous process of the lungs or other organs. Thus, Hertz (22), following 75 patients after exudative pleuritis with widespread pleural adhesions in the region of the diaphragm and the mediastinum, could note a significant reduction in vital capacity, maximal ventilation, and arterial oxygen saturation.

Analogous results were obtained by Bucher and Gloor (10) and also by Bruce (13) in patients in whom it was necessary to discontinue pneumothorax due to pneumopleuritis.

Bronchographic studies of Hertz and his associates (23) in 48 patients with pulmonary tuberculosis attended by significant adhesions showed that the basic function is accomplished by the healthy lung. These findings compel a very cautious approach to the question of major surgical operations on the lungs in the presence of massive adhesions of the pleura.

Köster and Lent (24) studied the dependence of roentgenographically determined changes of the pleura on the degree of disturbance of respiratory function of the lungs in 50 patients with pleural adhesions after exudative pleurisy and artificial pneumothorax. It was established that respiratory impairment is expressed less in changes of the pleurae in the upper and lateral areas than with basal adhesions.

Köster (25) studied respiratory function in 50 patients with massive pleural adhesions after pneumothorax and exudative pleuritis with ordinary breathing and breathing in an atmosphere of oxygen. In the majority of the patients a hidden respiratory insufficiency was revealed at rest as well as a decline in the vital capacity of the lungs. However, with comparatively satisfactory indices of the vital capacity and of reserve air, a rather significant reduction in the maximal ventilation was noticed. The writer explains this as a disturbance of respiratory mechanics. In young patients (up to thirty years), the impairment of mobility of the diaphragm in connection with pleural adhesions is compensated by the elasticity of the thoracic tissues, which are unable to change in older patients. After a course in respiratory exercises, a moderate elevation (of 6 to 10 per cent) was noted in the vital capacity and maximal ventilation.

Hence, the unfavorable influence of pleural adhesions on respiration depends on the localization, the massiveness of the adhesions, and the age of the patient.

The influence of emphysema on respiration: At the present time studies are being undertaken on the early diagnosis of emphysema, since it limits, to a great extent, the possibility of surgical intervention.

Curtis and associates (26) believe that the increase of residual air is not an early sign of emphysema. For the determination of emphysema at an early stage of its development, the writers propose repeated assessments of the vital capacity with the spirogram of the reduced maximal ventilation and of the content of nitrogen in the alveolar air upon breathing in an atmosphere of pure oxygen. For the diagnosis of emphysema, Anderson and associates (27) propose the use of the index of alveolar-arterial oxygen tension (in emphysema, it is increased).

For the purpose of improving respiratory function in emphysema, Faradic stimulation of the thoracic musculature and of the diaphragm, respiratory exercises, and appropriate medications are used.

Becklake and associates (28) conducted a rather intensive study of respiratory function in 15 patients with chronic hypertrophic emphysema prior to, during, and after the use of a monthly series of physical exercises for the stimulation of expiration both with simultaneous use

of the Faradic current and without it. The spirometric data, minute and maximal volumes of respiration, oxyhemometric indices, reserve base of the blood, and electrocardiographic indices did not improve after the application of these measures. A subjective improvement was attributed by the writers to the influence of the exercises on the psyche of the patient.

Campbell and Friend (29) also studied the influence of physical exercises on the respiratory function and the electrocardiographic indices of 12 patients with chronic emphysema. The observations compiled over the course of six months showed that after physical exercises the frequency of respiratory movements is decreased and the depth increased, the expiratory breathing is prolonged, and true lung capacity is increased, due to an increase of the activity of the abdominal muscles. The effectiveness of mixing of gases in the lungs and the volume of the dead space do not change with this. Despite the favorable results obtained, the writers do not draw final conclusions, alluding to the brief duration of their observations.

For improving respiratory function in emphysema and chronic bronchitis, a number of medicinal measures have also been proposed, possessing, primarily, bronchodilating properties. The effectiveness of the measures employed is determined by the degree of reduction in energy expenditure in the process of breathing (30).

Cherniack (31) recommends the use of broncho-aspiration in cases of unsuccessful treatment of chronic emphysema with manifestations of respiratory insufficiency. The suctioning off of gas through the broncho-aspirator in the course of thirty minutes elevates the oxygen tension, lowers the carbon dioxide tension in the arterial blood, and raises the reduced pH of the blood.

New Methods of Studying Respiration

The growth of pulmonary surgery has posed for the investigator the question of the separate study of the respiratory function of each lung. Presently existing methods of bronchospirometry do not satisfy the clinicians; thus, a number of improvements of this method have been proposed, and also the first efforts to study respiratory function in each lobe of the lungs have been undertaken. Studying the possibility of functional disturbances following pneumonectomy, Rossier (2) recommends studying, prior to operation, the function of the healthy lung by bronchospirometry, with lightening of the weight of the bell (to 150 gm.).

Jacob (32) believes it necessary to conduct bronchospirometry not only of the diseased lung but also of the healthy lung, prior to serious intervention, for the determination of the real functional potentialities of the remaining pulmonary parenchyma.

Bolt and associates (9) give a description of a bronchospirograph with two pumps and two spirometers, with the use of which they studied gas exchange and oxygen deficit at rest and under physical stress. They compared the findings obtained with breathing in common air with breathing in an atmosphere of pure oxygen.

Inada and associates (33) showed that bronchospirometric studies with the aid of a metallic Norris catheter are highly disturbing to the patient and hence give elevated results. Parallel studies of these same patients with a two-channel resin Carlens catheter showed less deviation from the normal at rest. Experiments with the use of physical effort (on the bicycle ergometer) showed that the uptake of oxygen increased to an identical degree in the more and in the less damaged lung. The writers believe that the results of their experiments disprove the general opinion that a damaged lung at rest is reaching the limits of its functional potential with respect to its uptake of oxygen.

At the present time, wide use is being made of the method of determination of the concentration of nitrogen in the respired air upon breathing in an atmosphere of oxygen. For the determination of the ventilation of a lobe, Martin and Young (34) used a study of the curve of the nitrogen breathed by the lobe. The latter was qualitatively similar to the curve of ventilation of the studied lobe. Such a method allows establishing how quickly the alveoli are cleared at expiration. The writers were able to establish the fact that the ventilation within the lobe is uneven. Upon comparing the ventilation the upper and the lower lobes, it was noted that, if the patient is in the horizontal position, the respiration of nitrogen of both lobes is identical; whereas, in the vertical position, the ventilation of the lower lobe is the more effective. Certain variations observed in the concentration of the respired nitrogen, which were synchronous with the work of the heart, were noticed and were attributed con-

jecturally by the writers to either the pressure of the heart on the adjacent lobe or the increase of the pulse pressure within the pulmonary artery.

The unequal distribution of ventilation in the lungs was regarded as one of the causes of deterioration in gas exchange. However, this could not be determined in a practical fashion. The method of determination of the concentration of nitrogen in the respired air with respiration in an atmosphere of oxygen allowed Ulbrich and Luft (35) to demonstrate a significant irregularity in the distribution of ventilation in the lungs in the development of different types of respiratory disturbances. The writers studied 150 patients with diverse diseases of the organs of respiration and 15 healthy persons.

In so far as the manometric method of studying blood gases by the Van Slyke technique is complicated and burdensome, a number of new methods of study have been proposed, among them the spectrographic method. In this method, as well as in the Van Slyke manometric method, the blood from an artery or vein is taken under oil and investigated in a special apparatus with a graduated scale. Wade and associates (36) conducted parallel studies of the oxygen saturation of the blood by manometric and spectrographic methods in 30 healthy people. In comparing the findings they obtained only very slight differences (approximately 0.1 per cent). The writers describe in detail the method of study and recommend it for extensive use in the clinic. This method indeed deserves attention, for, due to its simplicity, it provides an opportunity for wide use of the analysis of blood gases.

Opitz (37) indicates the possibility of a practical application of the hemoxytensiometer of Borrel and Levy for the determination of the alveolar-arterial difference in oxygen pressure which, to a definite extent, characterizes the process of uptake of oxygen in the lungs.

At the present time it has become possible to study the status of the lesser circulation by means of catheterization of the pulmonary artery, partial angiography of the finer radicals of the pulmonary arteries, the use of the method of labeled atoms by the introduction into the blood of erythrocytes emitting gamma-rays, and the determination of their circulation in the lesser and greater divisions of the blood circulation (8, 38).

Bolt (38) and Knipping (8), employing catheterization of the pulmonary artery in 1,650 patients and segmental angiography in 500 patients with tuberculosis of the lungs, affirm that these methods are completely innocuous.

Knipping (8) indicates that the use of the above methods along with the common ones (spirometry, spiro-ergometry, and study of the blood gases) permits the determination of cardiac insufficiency at rest and with effort, of the presence of constrictions in the vessels of the lesser circulation, of a change in the volume of the dead space, and of disturbances of the diffusion of gases in the alveoli.

The measurement of pressure in the pulmonary artery and in the right heart showed that, in cases of pulmonary tuberculosis without secondary pulmonary emphysema, the pressure in the lesser circulation is not elevated (38). The same writer, on the basis of spirometric studies at rest and with effort, studies of the blood gases, catheterization of the right heart and of the pulmonary artery, selective angiography and the use of the method of labeled atoms, established the following:

In pulmonary tuberculosis, there is a change in the relationship of the pulmonary ventilation and the pulmonary circulation in connection with the changes in the parenchyma of the lungs, consisting of a diminution in the number of lung vessels and constrictions of their lumina which quite distinctly appear upon selective angiography. Pneumothorax exerts a fundamental influence on the respiratory function as a consequence of the collapse of a large number of alveoli lying directly under the visceral pleura; in this there is compression of the vessels at the periphery of the entire lung and not only of the damaged part. Pneumolysis with extrapleural pneumothorax is the gentler method in a functional sense, for with this there is a collapse only of the damaged portion of the lung. In other regions of the lung on the side of the extrapleural pneumothorax, normal circulation and ventilation are preserved. Thoracoplasty elicits a more profound functional disturbance due to the compression of a large number of vessels. After lobectomy and pulmonectomy, emphysema ensues in the preserved portions of the lungs.

Hence, the use of the contemporary methods of study of the respiratory functions resolves, and at the same time poses, a number of complicated problems in the pathophysiology of respiration.

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ON THE ROLE OF THE THYROID IN NATIVE RESISTANCE TO TUBERCULOSIS[1, 2]

I. The Effect of Hyperthyroidism

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Introduction

It has been demonstrated by many observers that the alteration of the hormone balance which results from the administration of certain amounts of cortisone and its variants markedly lowers the resistance of many species, including man, to a variety of bacterial, viral, rickettsial, and parasitic infections (1). This would suggest that the underlying patterns of resistance to infectious diseases in general share certain dominant factors which may be influenced by hormones. In tuberculosis of inbred rabbits of uniform genetic resistance to the disease, it was found that pharmacologic doses of cortisone deprived the phagocytic mononuclears of much of their innate capacity to inhibit the growth of the bacilli in their cytoplasm (2). An analysis of the differences in the host-parasite relationships in natively resistant and susceptible rabbits (3) demonstrated that in the first few days of the infection the inhibition of intracellular bacillary accumulation is much greater in the resistant than in the susceptible animal, long before specific acquired immunity is apparent. Manifest acquired immunity develops simultaneously in both races but its level is superimposed on, and quantitatively determined by, the original native resistance. Since it is clear that certain hormone changes can reduce native resistance, it is possible that other appropriate hormone alterations may enhance resistance. This is so if it be assumed that, among the constitutional factors which determine native resistance or susceptibility to infection, the hormone spectrum characteristic of each race may be a significant factor. An endeavor was therefore made to find an altered hormone level which would increase the native resistance of our inbred races.

In past studies in this laboratory on the mechanism of acquired immunity to tuberculosis, it was found (4*a*) that effective immunity to tuberculosis, which depends primarily on the increased inhibition of multiplication of the reinfecting microorganism in the mononuclear phagocytes, is associated with a nonspecific increment of the physiologic activity of these same cells, as evidenced by their increased phagocytic avidity for nonspecific particles and their increased propensity for division in response to such stimuli. Since thyroid hormones increase the metabolism of cells and accelerate growth and differentiation, it seemed possible that the native resistance to tuberculosis might be increased in certain races by increasing the amount of thyroid hormones available to the tissues and, conversely, that the native resistance of such races could be diminished by reducing the concentrations of these hormones.

The present paper records the effects of hyperthyroidism induced by L-triiodothyronine or L-thyroxine on the pathogenesis of tuberculosis. The succeeding study (4*b*) describes the influence of hypothyroidism produced by the administration of propylthiouracil or thyroidectomy on this process as well as an attempt to determine, to some degree, the mode of action of the resulting altered hormone states on native resistance. A preliminary report of these studies was published in 1956 (5).

Materials and Methods

Animals used: Approximately 350 rabbits were used in these experiments. These belonged to eight different races of different genetic resistance to tuberculosis and of varying degrees of inbreeding. In any one trial, the effect of hyper- or hypothyroidism was tested on the same genetic strain with litter mates in the control and experimental

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groups. Hyperthyroidism was effected by the administration of the hormones intramuscularly for two to four weeks prior to infection followed by injections of the hormones daily or three times weekly during the course of the infection.

The hyper- or hypothyroidism was usually measured by determining the oxygen consumption during two hours. The rabbit was starved for a minimum of seventeen to twenty-four hours prior to the test. It was then placed in a snugly fitting open box in which the animal's movements were reduced to a minimum but in which it was under no stress. The box was sealed with water by means of a bell jar structure made of Plexiglass. The oxygen consumption was measured by the use of a Sanborn E-1-S metabolism apparatus. The oxygen consumption of normal rabbits, as determined by this method, was very close to the values given by Kleiber (6). The basal metabolic rate was determined before treatment was begun. This constituted the base line. The basal metabolic rate of these same animals was determined after hormone treatment or other experimental procedures shortly before challenge and, usually, on several occasions during the progress of the infection. In some experiments the protein-bound iodine was determined as a measure of the circulating thyroid hormones.

Measurement of resistance: In previous studies (3, 7), it was demonstrated that the number of primary pulmonary tubercles which resulted four to five weeks after a single quantitative inhalation of virulent human tubercle bacilli, H37Rv, was a direct and quantitative measure of the native resistance of a given race of inbred rabbits. The greater the resistance, the fewer the tubercles formed. Therefore, the number of inhaled tubercle bacilli required to yield one tubercle is a direct quantitative measure of the resistance to the inception of the disease. The size of these tubercles, their histologic character, their content of tubercle bacilli, and the extent of their caseation are a reflection of the resistance of the animal to the progress of the infection.

The effect of hyperthyroidism on the growth and destruction of the bacilli in the tissue was further determined by quantitative culture of the lung, tracheobronchial lymph nodes, and spleen of control and experimental animals at different intervals following simultaneous exposure to the inhalation of known numbers of tubercle bacilli.

Allergic and nonspecific irritability: The effect of different thyroid states on the nonspecific inflammatory irritability of the tissues was measured by the intracutaneous injection of india ink particles or small amounts of turpentine. The degree of the resulting inflammation was correlated with the intensity of the tuberculin reactions observed at different intervals during the course of the infection. The allergic sensitivity was also correlated with the extent and character of the tuberculous process at the time of death.

Experimental Observations

The Effect of L-Triiodothyronine on the Growth of the Bacilli in the Lungs and Their Spread to the Draining Lymph Nodes

Twenty-four rabbits of the AD race of intermediate resistance to tuberculosis were divided equally into control and experimental groups. Ten of the twelve pairs were litter mates. The initial oxygen consumption of each rabbit was determined. This averaged 39 cc. of oxygen per minute per kg. to the $\frac{3}{4}$ power ($kg.^{3/4}$) in the control group, and 40 cc. in the group reserved for triiodothyronine treatment. The experimental animals were then subjected to thirty days of intramuscular triiodothyronine treatment, as recorded in table 1. The controls were given the solvent of the hormone in the same amounts, at the same intervals, and by the same route as the hormone-treated animals. The experimental and control rabbits were then each divided into three groups of 4 each, designated AD1, AD2, and AD3. The basal metabolic rate of each of the 24 rabbits was again determined zero to six days before they were exposed in groups of 4 (2 control and 2 experimental animals) to the quantitative inhalation of H37Rv. Treatment of the control and experimental groups was continued after infection, as indicated in the table. The simultaneously exposed rabbits were then killed one, seven, fourteen, and thirty days after infection. The basal metabolic rate of some of the control and experimental rabbits was also determined during the course of the infection.

No significant change in oxygen consumption took place in the normal rabbits before or after infection. However, the basal metabolic rate of the triiodothyronine-treated rabbits prior to infection had increased from an average of 40 cc. of oxygen per minute per $kg.^{3/4}$ to one of 54 cc., an increase of 35 per cent. The average increase in basal metabolic rate for these rabbits throughout the course of the entire experiment was practically the same. The experimental animals were thus definitely in a hyperthyroid state as gauged by their basal metabolic rate. It will also be noted in table 1 that the experimental rabbits lost an average of 11 per cent of their body weight as a result of the triiodothyronine treatment. The control, solvent-treated animals lost less than 4 per cent.

The fate of the bacilli in the lungs of the control and experimental rabbits is recorded in table 2. The exact procedures used were described in a previous communication (3). The control rabbits inhaled 8,000 to 18,000 isolated bacilli; the hyperthyroid rabbits inhaled 10,000 to 20,000 micro-

TABLE 1

THE BASAL METABOLIC RATE OF TRIIODOTHYRONINE-TREATED "AD" RABBITS BEFORE AND DURING INFECTION

(in Cc. of Oxygen Consumed per Minute per $Kg.^{2/4}$)

Rabbit Number	Group	Before Treatment	After Treatment; Day Before Infection in ( )	After Infection; Day of Infection in ( )	Per Cent Change in O_2 Consumption	Per Cent Change in Weight Before Infection	Duration of Infection in Days
AD 5=24	AD-1	39.8	55.5 (5)	—	+39	−6	1
AD 5=22	AD-2	35.0	55.8 (1)	—	+60	−29	1
AD 5=31	AD-3	39.2	56.0 (5)	—	+43	−16	1
AD 5=35	AD-1	36.6	45.0 (3)	—	+23	−9	7
AD 5=40	AD-2	44.8	53.6 (2)	—	+20	−8	7
AD 5=32	AD-3	37.2	55.9 (5)	—	+50	−13	7
AD 5=52	AD-2	46.4	58.5 (2)	—	+26	−7	14
AD 5=51	AD-3	41.4	64.1 (5)	—	+55	−12	14
AD 5=51	AD-3	—	—	50.0 (9)	+21		
AD 5=43	AD-1	41.8	50.0 (3)	—	+20	−8	30
AD 5=43	AD-1	—	—	49.1 (22)	+17		
AD 5=43	AD-1	—	—	58.2 (29)	+39		
AD 5=26	AD-2	40.8	52.1 (1)	—	+28	−10	30
AD 5=26	AD-2	—	—	46.5 (14)	+14		
AD 5=26	AD-2	—	—	52.8 (28)	+29		
AD 4=20	AD-3	37.0	53.0 (5)	—	+43	−9	30
AD 4=20	AD-3	—	—	51.0 (8)	+38		
AD 4=20	AD-3	—	—	52.3 (28)	+41		
Average.....		40	54	51	+34	−11	

Treatment Schedule

(40 γ L-triiodothyronine intramuscularly)

AD-1	*AD-2*	*AD-3*
Alternate days for 3 days	Alternate days for 3 days	Alternate days for 3 days
Daily for 14 days	Daily for 15 days	Daily for 15 days
Alternate days for 13 days	Alternate days for 12 days	Alternate days for 12 days
Infection	*Infection*	*Infection*
Alternate days for 23 days	Alternate days for 14 days	Alternate days for 7 days
Daily for 7 days	Daily for 14 days	Daily for 21 days

organisms evidently because of their increased basal metabolic rate. In all instances, in calculating the rate of accumulation of the bacilli in the lungs, a third of the bacilli inhaled were considered to be seeded in the lungs (7–9). The number of bacilli recovered from both lungs is given both on direct culture and after treatment with sulfuric acid. As previously noted (3), this treatment reduced the number of bacilli recovered from the tissues by a constant multiple of 2 to 3.

A comparison of the actual numbers of tubercle bacilli recovered from the lungs of the hyperthyroid and the control rabbits at different intervals after infection shows a marked reduction of the population in the triiodothyronine-treated rabbits both on direct culture and after treatment. A clearer indication of the marked inhibition of growth of the microorganism in the hyperthyroid rabbits may be obtained by a comparison of the four last columns of table 2. Thus, seven days after infection the bacilli in the lungs of the control rabbits constituted 70 per cent of the number seeded. In the hyperthyroid rabbits the bacilli were only 8 per cent of this number; the inhibition of growth was thus 10 times greater in the experimental animals. Fourteen and thirty days after infection, this suppression was 44 and 22 times greater, respectively, in the hyperthyroid animals. Similar differences were obtained between these two groups when treated cultures were compared.

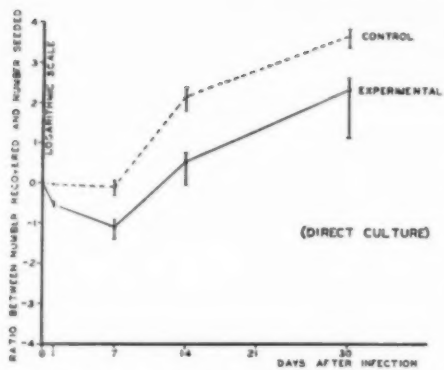
The population changes in the two groups are

TABLE 2

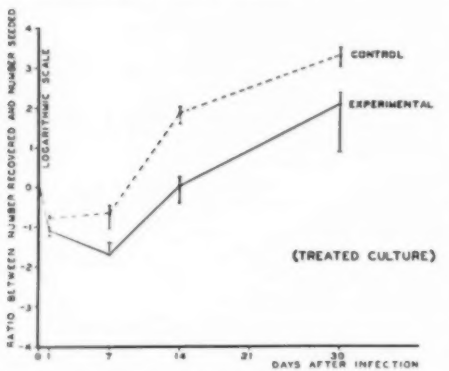
THE RATE OF CUMULATION OF HUMAN TUBERCLE BACILLI IN THE LUNGS OF CONTROL AND TRIIODOTHYRONINE-TREATED AD RABBITS AT DIFFERENT INTERVALS FOLLOWING SIMULTANEOUS INHALATION OF H37Rv

Days After Infection	Rabbit Number		Average Per Cent Change in Q_{O_2}*		Number of Bacilli Estimated as Seeded in Lungs		Number of Viable Bacilli Recovered from Lungs				Ratio Between Number Seeded and Recovered			
							Direct		Treated		Direct		Treated	
	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal
1	5=23	5=24	+8	+40	4,132	6,605		2,329	492	476		0.35	0.12	0.07
	5=29	5=22	+1	+60	3,609	3,428	3,366		752	370	0.93		0.21	0.11
	5=30	5=31	+15	+43	2,681	3,561		900	448	210		0.25	0.17	0.06
	Average or mean		+6	+48								0.30± 0.05	0.17± 0.03	0.08± 0.02
7	5=37	5=35	+8	+23	5,426	5,498	7,784	810	2,601	405	1.44	0.15	0.48	0.07
	5=39	5=40	+6	+20	3,269	3,950	1,680	149	471	0	0.51	0.04	0.14	0.0
	5=41	5=32	+7	+50	3,013	3,776	1,251	144	153	0	0.42	0.04	0.05	0.0
	Average or mean		+7	+31							0.79± 0.33	0.08± 0.04	0.22± 0.13	0.02± 0.02
14	5=53	5=52	+13	+26	2,960	3,493	173,330	3,030	121,440	1,516	59.0	0.87	41.0	0.43
	5=54	5=51	+9	+38	4,598	6,835	1,034,800	37,500	492,100	12,380	225	5.5	107	1.8
	Average or mean		+11	+32							142± 83	3.2± 2.3	74± 33	1.7± 0.7
30	3=51	5=43	−2	+26	5,973	6,188	53,283,300	9,800	25,344,200	19,600	8,920	1.6	4,243	3.1
	4=11	5=26	+16	+24	3,473	3,197	4,463,300	100,400	1,441,900	47,500	1,285	31.4	415	15.0
	4=19	4=20	+7	+41	4,101	5,437	15,540,000	3,172,000	7,992,000	2,014,000	3,789	583	1,949	370
	Average or mean		+7	+30							4,665± 2,290	205.3± 192	2,202± 1,132	129.4± 122

* During course of experiment as compared to initial pretreatment value



TEXT FIG. 1. The effect of L-triiodothyronine on population changes of human bacilli (H37Rv) in the lungs of AD rabbits at different intervals after quantitative air-borne infection.



TEXT FIG. 2. The effect of L-triiodothyronine on population changes of human tubercle bacilli (H37Rv) in the lungs of AD rabbits at different intervals after quantitative air-borne infection.

plotted on a logarithmic scale in text figures 1 and 2. As was found in a previous study in untreated resistant race III and susceptible race C, there is first a lag phase in growth, then a phase of rapid multiplication followed by a period of less rapid growth. Since no cultures were made at three weeks after infection, the impression was gained that there was only a slowing in growth in the interval between fourteen and thirty days, as compared with the interval between seven and fourteen days. As will be seen in a later portion of this report, when the effect of L-thyroxine on the multiplication of the bacilli was considered, active growth ceased at the end of the third week. Despite this defect, it was evident that the inhibition of growth in the hyperthyroid animals appeared very early, and was marked seven days after infection. This increased inhibition continued throughout the course of the infection in the animals under the influence of triiodothyronine.

It will be remembered (3) that one fundamental difference in behavior between an untreated natively resistant and a natively susceptible animal is the greater immediate and continued inhibition of bacillary accumulation in the resistant animal. Triiodothyronine in AD rabbits has the same effect.

Another similarity between the effect of triiodothyronine treatment and the behavior of resistant rabbits relates to the spread of bacilli from the lung to the draining tracheobronchial lymph nodes. It may be recalled that, contrary to expectation, it was found that this spread was greater in the resistant than in the susceptible animal (3). In table 3, the ratio between the number of viable bacilli per unit weight of draining lymph nodes and a unit weight of lung at different intervals following inhalation is presented in control and triiodothyronine-treated rabbits. The tracheobronchial lymph nodes were free of tubercle bacilli in both groups one day after infection. Seven days after exposure the lymph nodes of 2 of the 3 rabbits in both groups were still sterile. The lymph nodes of one of the rabbits of each group, however, contained tubercle bacilli. It is noteworthy that, while the bacilli in the lymph nodes of this control animal constituted about 5 per cent of those in the lung, 36 per cent of the bacilli in the lungs were recovered from the draining lymph nodes of the corresponding hormone-treated animal. In 4 of the 5 remaining rabbit pairs, the percentage of pulmonary bacilli present in the draining lymph nodes of the treated animals far exceeded those in the lymph nodes of the control rabbits. It is thus clear that the bacilli in the lungs of the hormone-treated animals are swept to the draining lymph nodes more effectively than in the untreated rabbits of the same genetic resistance. This interpretation is based on the assumption that the bacilli found in these lymph nodes are largely derived from the region which they drain, namely the lung.

The Suppression of Tuberculosis in AD Rabbits by L-Triiodothyronine

Since only one lung was cultured quantitatively in the intervals of fourteen and thirty days after infection, the other lung was weighed and studied

TABLE 3

THE RATIO BETWEEN THE NUMBER OF BACILLI IN THE TRACHEOBRONCHIAL LYMPH NODES AND IN THE LUNG, PER UNIT WEIGHT OF EACH, IN PER CENT

	Control Animals				Experimental Animals			
Days After Infection	Rabbit Number	Number of Bacilli in 1 gm. of Lung Tissue	Number of Bacilli in 100 mg. of Lymph Node	Percentage of Bacilli in Lung, Recovered from Lymph Node	Rabbit Number	Number of Bacilli in 1 gm. of Lung Tissue	Number of Bacilli in 100 mg. of Lymph Node	Percentage of Bacilli in Lung, Recovered from Lymph Node
1	AD 5=23	—	0	0	AD 5=24	284	0	0
	AD 5=29	391	0	0	AD 5=22	—	0	0
	AD 5=30	—	0	0	AD 5=31	125	0	0
7	AD 5=37	846	42	4.9	AD 5=35	100	36	36
	AD 5=39	179	0	0	AD 5=40	15	0	0
	AD 5=41	136	0	0	AD 5=32	15	0	0
	Average...			1.6	Average...			12
14	AD 5=53	19,696	12,800	65	AD 5=52	333	350	105
	AD 5=54	108,926	2,100	1.9	AD 5=51	3,788	8	0.2
	Average...			33.4				52.6
30	AD 3=51	3,833,306	12,000	0.3	AD 5=43	1,000	36	3.6
	AD 4=11	433,333	1,060	0.2	AD 5=26	13,044	12,100	93
	AD 4=19	2,100,000	50	0.002	AD 4=20	406,667	882	0.2
	Average...			0.2	Average...			32.3

TABLE 4

THE EFFECT OF L-TRIIODOTHYRONINE ON TUBERCULOSIS IN AD RABBITS THIRTY DAYS AFTER THE QUANTITATIVE INHALATION OF H37RV EXPERIMENT OF NOVEMBER AND DECEMBER, 1955

Rabbit Number	Number of Tubercle Bacilli Inhaled, Estimated on Basis of BMR* before Infection	Percentage Change in O_2 Consumption During Infection	Tuberculin Sensitivity in mm.[5]	Number of Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Size of Tubercles	Caseation
Control Animals						
AD 3=51	17,920	−2	480	129	4.5	Extensive
AD 4=11	10,419	+16	486	316	3.6	Extensive
AD 4=19	12,302	+7	184	267	3.8	Extensive
Average.....		+7	383	237	4.0	
Triiodothyronine-treated Animals						
AD 5=43	18,564	+26	58	6,188	1.0	None
AD 5=26	9,590	+24	58	>9,590	Microscopic	None
AD 4=20	16,310	+41	34	1,359	2.0	Slight
Average.....		+30	50	5,712	1.5	

* Basal metabolic rate

grossly and microscopically. In table 4 the pertinent data are recorded relative to the 3 experimental and the 3 control rabbits killed thirty days after inhalation. The tuberculosis in one lung of each of these 6 rabbits is depicted in Plate I, figure 1; the other lung of each rabbit was cultured and is discussed above. It must be stated that the exact number of tubercles in the lungs used for culture could not be accurately determined. Therefore, the number of tubercles in

these lungs was estimated on a weight basis from the number of tubercles formed in the weighed lungs reserved for pathologic study, in which enumeration of the tubercles was possible.

As is evident from figure 1 of Plate I, the tuberculosis in the triiodothyronine rabbits was largely suppressed. In one of these experimental rabbits, the suppression was incomplete. In column 5 of table 4, it may be seen that an average of 237 inhaled bacilli was required to generate one tubercle in the control rabbits. In the experimental animals, 5,712 microorganisms were required to produce one focus. The resistance of the treated rabbits on this basis was, therefore, increased about 24 times. It is interesting that the inhibition of bacillary accumulation in these 3 treated rabbits averaged about 22 times greater than in the 3 control rabbits, which is of the same order of magnitude as the increment in native resistance (*see* table 2, columns 12 and 13 for the thirty-day interval). Not only did this increased inhibition of growth afforded by the triiodothyronine treatment suppress the inception of the tuberculosis, but it also markedly inhibited the progression of the tubercles formed. The tubercles in the control rabbits were large, with extensive caseation and numerous bacilli within the immature epithelioid cells. Those in the experimental animals were minute collections of mature epithelioid cells with rare, short bacilli surrounded by masses of lymphocytes and round cells. There was little or no caseation. The suppression of bacillary growth by triiodothyronine and the characteristic tissue response is illustrated in Plate II, figures 4 and 5.

Another observation that deserves emphasis is the marked suppression of the intensity of the tuberculin sensitivity in hormone-treated rabbits. This sensitivity in the control rabbits was 7 times greater than that in the experimental animals and was apparently associated with the far greater bacillary population in the lungs of the former.

The Effect of Triiodothyronine on an Already Existing Tuberculosis

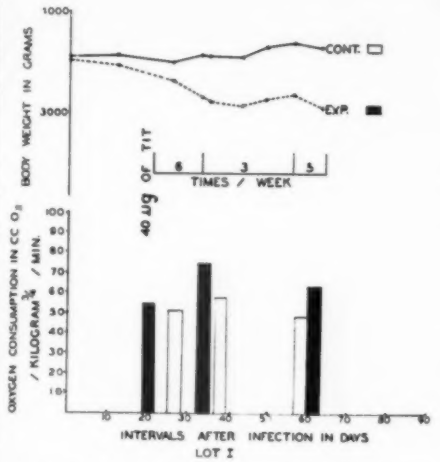
Since it was found that L-triiodothyronine treatment not only suppresses the inception of the disease but also retards its progress, it was decided to determine whether this hormone would affect an already existing tuberculosis.

A group of 22 litter mates of the first and second inbred generations of a recently developed family, III IIIC[4] was simultaneously exposed, in two lots, to the quantitative inhalation of H37Rv in February, 1956. Nineteen days afterward all of the rabbits showed roentgenographic evidence of tuberculosis and all reacted to tuberculin. The rabbits were then divided into two groups consisting of equal numbers of simultaneously exposed litter mates. As, in the early stages of this type of tuberculosis, the intensity of the tuberculin reaction is greater the more extensive the disease, the litter mates that reacted with greater inflammation were selected for triiodothyronine treatment to be certain that the animals to be treated with the hormone had at least as extensive a disease as that in the controls. Triiodothyronine therapy was begun twenty-one days after infection. The control animals were treated with the solvent in which the hormone was dispensed at the same intervals, by the same route, and in the same volumes.

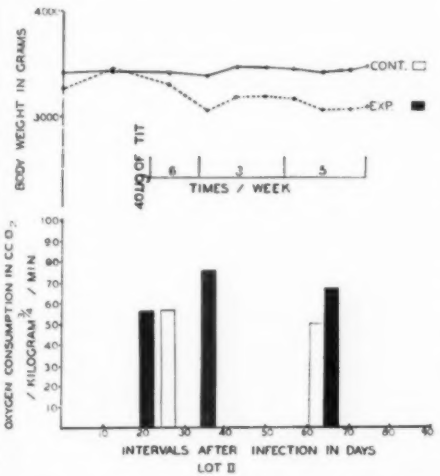
In text figures 3 and 4 are set forth the treatment regimen, the changes in basal metabolic rate, and the changes in weight during the course of the experiment in the hormone-treated and control rabbits of lots 1 and 2, respectively. As may be seen from these figures, the oxygen consumption of the experimental groups before hormone treatment was begun averaged 54 and 56 cc. of oxygen[5] per minute per kg.$^{3/4}$, respectively, for each lot. After triiodothyronine treatment, this average rose by approximately 29 to 30 per cent. The basal metabolic rate of the solvent-treated, control rabbits did not change significantly during the course of the experiment, nor did it vary beyond the error inherent in the method from that of the rabbits in the experimental group before triiodothyronine treatment. The experimental rabbits in both lots tended to lose weight during the first two weeks of treatment, when 40 γ of triiodothyronine was given six times a week. When this amount was given three times a week, no further loss in weight occurred. When this hormone was again increased to five times a week, in the latter part of the experiment, there was a tendency to additional weight loss. At no time, however, did this loss exceed 10 to 11 per cent in either lot. The control, solvent-treated rabbits, on the other hand, maintained their

[4] This race was derived from a cross between the resistant race III and the susceptible race C, backcrossed to the resistant race III (10).

[5] The cc. of oxygen consumed by rabbits in this single experiment, as determined by a certain model of E-1-S machine, were higher than in any other done before or since. This particular, apparently faulty, apparatus was never used again.



TEXT FIG. 3. The body weight and oxygen consumption of III IIIC rabbits with and without triiodothyronine treatment begun three weeks after quantitative inhalation of H37Rv.



TEXT FIG. 4. The body weight and oxygen consumption of III IIIC rabbits with and without triiodothyronine treatment begun three weeks after quantitative inhalation of H37Rv.

weight or gained slightly, 2 to 3 per cent, during the entire course of the experiment.

Since the response of the experimental and control rabbits of lots 1 and 2 did not differ significantly, they are considered together in table 5.

It will be seen that the tuberculin sensitivity on the nineteenth day after infection was greater in 10 of the 11 rabbits of the group subjected two days later to triiodothyronine treatment. Therefore, as considered above, one may assume that the extent of the tuberculosis in the experimental group before hormone treatment was begun was at least equivalent to that in their litter-mate controls. Lot 1 of both groups was exposed to the inhalation of 33,000 to 53,000 bacilli. Those of lot 2 inhaled 127,000 to 156,000 microorganisms. Despite this three- to fourfold difference in the infecting dose, and notwithstanding the heterogenicity of the two lots, there was a two- to threefold reduction in the number of residual tubercles per unit of inhaled bacilli in the treated as compared with the control litter mates when killed sixty-three to seventy-five days after infection. Considering the two lots together, it was found that, in the controls, 359 plus-minus 64 inhaled bacilli yielded one tubercle. In the experimental rabbits 1,004 plus-minus 371 microorganisms (or 2.8 times as many inhaled bacilli) were required to produce one tubercle.

The resistance of the experimental group was thus increased two- to threefold. Similarly, the tubercles in the hormone-treated animals were smaller. The difference between these observations is statistically significant at the 5 per cent level. This difference is illustrated in Plate I, figure 2. It will be noted in column 6 of table 5 that in the experimental group there is one rabbit, III IIIC1-59, with an unusually high resistance, 4,657. If this observation is omitted and the litter-mate control, rabbit III IIIC1-57, with the highest resistance in the control group, 812, is also omitted, the relative resistance of the experimental rabbits becomes 639 plus-minus 75 as compared with 313 plus-minus 50 for the controls with a P value of the difference of 0.001. Again, even if the exceptionally resistant triiodothyronine-treated rabbit is eliminated and all of the controls are kept, the difference between the two groups, although less in extent, would still be in the same direction and statistically highly significant. As further evidence of reliability of the observed difference, it may be stated that 12 untreated rabbits of the same race exposed to the inhalation of H37Rv in October, 1955, required 315 plus-minus 67 tubercle bacilli to yield one tubercle thirty-three days after infection. The resistance of these rabbits was, therefore, essentially the

TABLE 5

THE EFFECT OF TRIIODOTHYRONINE TREATMENT ON AN ALREADY EXISTING TUBERCULOSIS CAUSED BY THE QUANTITATIVE INHALATION OF H37Rv

Rabbit Number*	OT Reaction Before Treatment in mm.[a]	Duration of Treatment in Days	Number of Bacilli Inhaled	Number of Tubercles Formed	Number of Inhaled Bacilli Yielding 1 Tubercle *Resistance*	Size of Tubercles in mm.
Control Animals—Treated with Solvent						
III III C1-39 (a)	85	42	33,591	184	183	4.5
III III C1-51 (a)	400	42	37,205	315	118	4.0
III III C1-56 (b)	51	43	33,803	294	115	4.6
III III C1-57 (c)	58	43	48,759	60	812	2.0
III III C1-60 (c)	220	22	51,228	83	617	2.7
III III C2-20 (d)	251	44	53,079	161	329	2.7
III III C2-22 (d)	744	53	132,367	495	267	2.1
III III C2-26 (d)	88	53	139,955	447	313	2.7
III III C2-29 (d)	180	53	154,287	369	418	4.1
III III C2-34 (d)	104	54	156,138	499	313	3.6
III III C2-36 (d)	580	54	138,681	301	416	3.3
Mean	251				359 ± 64†	3.3 ± 0.3†
Experimental Animals—Treated with Triiodothyronine						
III III C1-40 (a)	166	42	36,142	172	210	3.0
III III C1-52 (b)	592	42	33,803	42	805	3.9
III III C1-59 (c)	220	43	51,228	11	4,657	1.6
III III C1-62 (c)	101	22	52,462	58	905	3.5
III III C2-21 (d)	420	44	49,685	54	920	3.3
III III C2-24 (d)	386	53	139,955	302	463	3.4
III III C2-27 (d)	210	53	134,053	341	393	2.5
III III C2-31 (d)	634	53	127,308	158	806	2.0
III III C2-33 (d)	700	54	143,530	232	619	1.5
III III C2-35 (d)	68	54	153,228	292	525	3.0
III III C2-37 (d)	264	54	143,530	193	744	2.4
Mean	369				1,004 ± 371†	2.7 ± 0.2†
12 rabbits‡			2,200–3,600	3–56	315 ± 67	4.1 ± 0.2

* Rabbits with the same letter are litter mates.
† P value of the difference between these respective means = 0.05.
‡ 12 III IIIC untreated rabbits exposed in October, 1955.

same as that of the control rabbits of February, 1956. Furthermore, it is also clear that the number of tubercles formed thirty-three days after infection did not diminish if similar untreated rabbits were similarly exposed and killed sixty-three to seventy-five days after inhalation infection. Therefore, it may confidently be presumed that no resolution of tubercles present thirty-three days after infection took place when the control rabbits of the present experiment were killed some forty days later. This is important in understanding the significance of the observed increment of resistance produced by triiodothyronine in a tuberculosis of twenty-one days' duration in this family.

It was found in CaC rabbits (5), as well as in the AD rabbits reported above, that triiodothyronine treatment instituted before infection and continued thereafter markedly suppressed the tuberculin sensitivity. However, in this experiment, triiodothyronine treatment begun three weeks after infection exerted no influence on the allergic sensitivity of the first lot originally exposed to approximately 30,000 to 50,000 bacilli. In the second lot, exposed to a three- to fourfold greater dose, there was a tendency for the tuberculin sensitivity to be reduced in the hormone-treated rabbits during the last four weeks of the disease. Since the differences are not statistically significant, they are not presented in detail. How-

ever, it is evident that mere triiodothyronine treatment per se does not reduce tuberculin sensitivity unless, as shown above in the AD rabbits, there is a concomitant profound suppression of the multiplication of the bacilli and, consequently, a very marked reduction in the extent of the disease.

The Effect of Triiodothyronine on the Very Susceptible Race C

It was not always possible to increase significantly the native resistance by the administration of L-triiodothyronine. As an illustration, the effect of such treatment on the native resistance of the highly inbred and highly susceptible race C is presented in table 6.

The treatment regimen was similar to that of the AD rabbits given above, with the following modifications.

The C rabbits as well as a few rabbits of another race, CaC, were treated with the hormone for twenty-seven days prior to infection. They received 40 γ intramuscularly on alternate days for nine days and the same dose six times a week for the remaining eighteen days prior to infection. After infection they were treated with 40 γ three times weekly throughout the course of the disease. The controls were injected with the solvent as in the previous experiments.

One to three days before challenge, the basal metabolic rate of the experimental group rose by an average of 42 per cent over their base line. During the course of the entire experiment this increase, as listed in table 6, averaged plus 32 per cent, which is of the same magnitude as that obtained in the AD and III IIIC rabbits, in which the hormone definitely increased resistance. However, the resistance of the hormone-treated rabbits of this race was only slightly increased over

TABLE 6

THE EFFECT OF TRIIODOTHYRONINE ON THE INCEPTION AND PROGRESS OF TUBERCULOSIS IN "C" RABBITS 31 TO 33 DAYS AFTER THE QUANTITATIVE INHALATION OF H37Rv

Rabbit Number	Percentage Change in BMR* over Base Line	Protein Bound Iodine in γ per 100 ml. of Blood	Number of Tubercle Bacilli Inhaled, Estimated by BMR	Number of Tubercles Formed	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Average Diameter of Tubercle in mm.	Type of Tubercle	Extent of Caseation	Maximal OT Sensitivity in mm.[5]
Control Animals									
C14=17	−4	7.1	13,456	345	39	4.1	Some spreading	Extensive	518
C14=46	+3	7.1	6,773	175	39	5.2	Spreading	Extensive	170
C14=36	+14	5.7	7,012	167	42	5.2	Some spreading	Extensive	176
C13= 4	0	7.2	14,970	281	53	4.2	Some spreading	Extensive	252
C14=33	+1	6.1	7,823	145	54	4.0	Spreading	Extensive	364
C14=34	−3	7.5	11,185	151	74	5.0	Spreading	Extensive	251
Average	+2	6.8			50 ± 6†	4.6 ± 0.3			289
Experimental Animals									
C14=25	+18	5.6	9,158	214	43	4.3	Some discrete	Prominent	1,126
C14=30	+48	4.5	8,825	170	52	5.2	Spreading	Moderate	87
C15= 7	+20	5.4	15,306	273	56	4.2	Spreading	Slight	250
C14=32	+22	5.7	9,302	138	67	3.5	Discrete	Slight	605
C14=49	+30	6.9	17,493	200	87	3.9	Mostly discrete	Slight	851
C15= 4	+54	4.9	16,904	104	163	4.3	Discrete	Slight	240
Average	+32	5.5			78 ± 18†	4.2 ± 0.1			526

* Basal metabolic rate

† P value of the difference between these observations = 0.08

that of the solvent-treated controls, both from the standpoint of the number of tubercles generated, their progression, and their histologic character. This difference was not statistically significant. Incidentally, the few hormone-treated CaC rabbits of low resistance included in this experiment (but not listed in the table) showed a fourfold increment in resistance over simultaneously exposed control rabbits of the same race.

It is noteworthy that the tuberculin sensitivity of the experimental C rabbits was, if anything, higher than that of the controls. Hence, mere hyperthyroidism does not diminish allergic sensitivity if it is not accompanied by a marked suppression of the tuberculosis.

It may be of significance to state here that in a preliminary experiment, with a small number of animals, the native resistance of the very highly resistant race III could not be further increased by triiodothyronine despite a 34 per cent increment of their basal metabolic rate. Therefore, it is not merely the additional L-triiodothyronine supplied that increases resistance. The interplay of host constitutional factors is obviously of decisive importance.

The Effect of L-Thyroxine on Native Resistance

In the studies hitherto reported, 1-3:5:3′ triiodothyronine has been used. It is well known that this substance is 2 to 5 times more active than L-thyroxine, as measured by its effect on the basal metabolic rate, its capacity to prevent goiter, its inhibition of I^{131} uptake by the thyroid, its suppression of pituitary thyrotropic activity, and its ability to promote metamorphosis (11). Therefore, it would not necessarily follow that thyroxine would have the same effect on native resistance as triiodothyronine. To answer this question and, if possible, to establish more fully the protective effect exerted by certain thyroid hormones on native resistance, additional studies were undertaken with L-thyroxine.

For this purpose 18 litter mates of the second and third inbred generations of the race IIIA of intermediate resistance to tuberculosis, were divided into two groups of 9 rabbits each. The experimental rabbits were given 100 γ of L-thyroxine intramuscularly three times a week for two weeks prior to challenge. This raised their basal metabolic rate by 44 per cent. The treatment was continued in the same dose, at the same intervals, and by the same route throughout the course of the disease. The control litter mates were treated with the solvent of the hormone in the same volumes and in an identical manner. Both groups were simultaneously exposed to the inhalation of 9,000 to 36,000 H37Rv bacilli and were killed thirty-three to thirty-five days after the infection. It may be seen in table 7 that the basal metabolic rate of the thyroxine-treated rabbits was increased by an average of 65 per cent during the infection. No consistent change in the basal metabolic rate of the control rabbits occurred during this time. It will be noted in column 5 that in the thyroxine-treated rabbits the tuberculosis was markedly and uniformly suppressed, as compared with that in the control litter mates. While for the control rabbits an average of 154 inhaled bacilli sufficed to yield one tubercle, 659 were required to produce one primary tubercle in the hormone-treated rabbits. The native resistance was thus increased fourfold. Furthermore, the progression of the tubercles formed in the thyroxine-treated rabbits was considerably retarded, as indicated by their small size and the slight extent of their caseation. These differences are statistically highly significant. The suppressive effect of L-thyroxine on the tuberculosis in this race is illustrated in Plate I, figure 3. As with triiodothyronine, the tuberculin sensitivity of the thyroxine-treated rabbits was markedly reduced. This fourfold difference is statistically highly significant.

There was no significant difference in the mass of the thyroid or the adrenal tissue between the control and experimental animals. However, the thyroxine-treated rabbits lost an average of 21 per cent of their body weight. This loss was confined to the fat depots which practically disappeared. There was no loss in muscle mass, and the spleens of the experimental rabbits were of the same weight as those of the control animals. However, calculated on a body-weight basis, the spleens of the experimental rabbits each weighed 65 mg. per 100 gm., whereas those of the control animals weighed 53 mg. Whether this indicates an hypertrophy of the spleen or a failure of atrophy of this organ concomitant with the loss of body fat is not immediately apparent.

Histologic study confirmed the macroscopic observations and indicated that in the thyroxine-treated animals the caseation was slight, punctate, often amorphous, and well advanced.

TABLE 7

THE EFFECT OF L-THYROXINE ON THE INCEPTION AND PROGRESS OF QUANTITATIVE INHALATION TUBERCULOSIS (H37Rv) IN RACE IIIA RABBITS. EXPERIMENT OF FEBRUARY 6–7, 1957
Duration of Disease 33 to 35 Days

Rabbit Number*	BMR† Per Cent Change During Infection	Weight Change at Death in Per Cent	Maximal OT Sensitivity in mm.[3]	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Size of Tubercle in mm.	Extent of Caseation
Control Animals						
3-6 (a)	+2	−2	470	118	3.8	Prominent
3-3 (a)	+3	−5	1,008	125	3.6	Moderate
3-1 (a)	−20	−11	826	117	3.9	Extensive
2-27 (b)	+5	+3	594	269	3.1	Moderate
3-12 (c)	+14	−2	868	184	2.8	Moderate
3-8 (c)	−6	0	753	287	3.2	Moderate
3-10 (c)	+2	0	1,056	157	3.3	Prominent
3-14 (d)	+17	−4	835	36	5.0	Extensive
3-15 (d)	−11	0	875	88	4.2	Prominent
Mean.....	<+1	−2	809 ± 62‡	154 ± 27§	3.7 ± 0.2‖	
Thyroxine-treated Animals						
3-5 (a)	+48	−26	158	472	2.1	Slight
3-2 (a)	+80	−19	136	953	2.2	None
3-7 (a)	+65	−9	181	997	3.0	Punctate
3-4 (a)	+87	−34	168	348	3.3	Punctate
2-26 (b)	+78	−26	202	840	2.4	Punctate
2-28 (b)	+59	−19	70	786	3.0	None
3-9 (c)	+90	−9	594	443	2.3	Punctate
3-11 (c)	+50	−19	168	898	3.1	Punctate
3-13 (d)	+30	−26	180	195	2.6	Slight
Mean.....	+65	−21	206 ± 50‡	659 ± 99§	2.7 ± 0.1‖	

* Rabbits with the same letter are litter mates.
† Basal metabolic rate.
‡ P value of the difference between these observations is <0.000.
§ P value of the difference between these observations is <0.000.
‖ P value of the difference between these observations is <0.006.

These wholly interstitial lesions contained isolated, rather short, poorly stained tubercle bacilli (Plate IV, figure 12). Extensive caseation, infiltrated with much nuclear debris in a less advanced state of autolysis with occasional pneumonic foci, characterized the tubercles of the control rabbits. They contained markedly larger numbers of bacilli in active growth, as indicated by the frequent occurrence of cords of long, deeply-stained, acid-fast bacilli (Plate IV, figure 11). The resolution of the tubercles and their permeation by capillaries and erythrocytes were further advanced in the hormone-treated than in the control rabbits (Plate IV, figures 13 and 14). Hyperplasia of the malpighian corpuscles of the spleen was evident in the majority of the experimental animals.

The Effect of L-Thyroxine on the Growth of the Bacilli in the Lungs and Their Spread to the Draining Lymph Nodes

To determine whether thyroxine suppresses tuberculosis by virtue of its inhibitory effect on the multiplication of the bacilli *in vivo*, as was shown above for triiodothyronine in the AD rabbits, a similar experiment was carried out with race III IIIC with and without thyroxine treatment.

Thirty litter mates were divided into three groups of 10 each. Five were treated with 100 γ of L-thyroxine intramuscularly three times a week for nineteen days; 5 were untreated controls. The control and experimental rabbits were then simultaneously exposed to the quantitative inhalation of H37Rv. This strain was recently obtained from Dr. William Steenken and evinced a

TABLE 8

THE RATE OF CUMULATION OF HUMAN TUBERCLE BACILLI IN THE LUNGS OF CONTROL AND L-THYROXINE-TREATED III III C RABBITS AT DIFFERENT INTERVALS FOLLOWING SIMULTANEOUS INHALATION OF H37Rv (STEENKEN)

Day After Infection	Rabbit Number		CC. of O_2 Consumed per Kg.$^{3/4}$		Number of Bacilli Estimated as Seeded in Lungs		Number of Viable Bacilli Recovered from Lungs				Ratio Between Number of Seeded and Recovered Bacilli			
							Direct		Treated		Direct		Treated	
	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal
1	3-33	4-8	44.5	68.5	861	1,286	910	2,150	341	430	1.06	1.67	0.40	0.33
	3-48	3-46	45.7	63.5	3,346	3,631	7,260	4,002	1,173	939	2.2	1.10	0.35	0.26
	4-9	4-5	43.4	65.0	10,246	16,205	8,973	6,854	1,750	1,305	0.88	0.42	0.17	0.08
	Average										1.38	1.06	0.31	0.22
7	3-34	3-30	45.2	61.0	965	1,298	5,662	—	2,293	792	5.87	—	2.38	0.61
	3-45	3-44	47.0	64.6	3,418	3,987	9,880	5,760	4,030	1,040	2.89	1.44	1.18	0.26
	4-3	4-1	42.8	66.3	10,827	16,277	110,075	5,647	80,500	2,420	10.16	0.35	7.44	0.15
	Average										6.30	0.90	3.67	0.34
14	4-4	4-2	45.2	56.4	983	1,153	912,000	75,333	651,700	51,792	927	65.3	663	44.9
	3-36	3-35	47.5	57.5	3,679	4,130	1,440,000	3,467,330	1,176,750	1,979,701	391	840.0	320	479.0
	4-11	4-10	38.1	78.8	9,301	17,803	18,228,000	1,080,000	5,899,600	810,900	1,960	60.7	634	45.5
	Average										1,093	322	539	190
21	3-32	3-31	47.3	58.0	743	931	22,041,000	7,742,250	9,439,500	1,633,676	29,655	8,316	12,704	1,754
	3-38	3-37	47.2	61.3	4,413	5,429	77,970,000	33,000,000	33,900,000	19,325,090	17,668	6,078	7,682	3,560
	4-7	4-6	44.3	78.8	2,819	4,443	87,033,000	32,330,000	62,794,480	21,630,000	30,874	7,277	22,275	4,868
	Average										26,069	7,224	14,220	3,394
28	3-41	3-40	43.4	59.3*	653	884	187,440,000	21,721,700	84,256,866	15,880,200	287,040	24,569	129,030	17,965
	3-43	3-42	43.3	71.0*	4,471	6,010	133,810,500	10,456,600	81,949,890	7,542,270	29,928	1,740	18,329	1,255
	3-49	3-47	46.0	61.5*	2,725	3,603	32,200,000	13,082,500	15,778,000	5,965,620	11,817	3,631	5,790	1,656
	Average		45.9	63.0*							109,595	9,980	51,050	6,958

* Average of two determinations

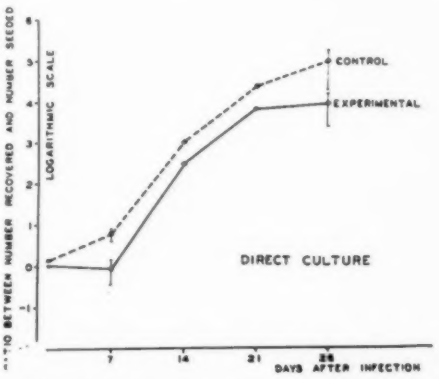
considerably higher virulence than the H37Rv used in the previous experiments. The thyroxine treatment was continued in the experimental rabbits at the same intervals and in the same dose throughout the course of the infection. One, seven, fourteen, twenty-one, and twenty-eight days after exposure, a control and a thyroxine-treated animal were killed and either both or one lung was quantitatively cultured. All of the tracheobronchial lymph nodes and the spleens were weighed and similarly cultured. This procedure was repeated on two more occasions until 6 rabbits, 3 controls and 3 experimental animals, were available for study for each of the five intervals.

It may be seen in table 8 that the basal metabolic rate of the thyroxine-treated rabbits had increased by 41 per cent over their base line, one to six days prior to challenge. No significant difference in the number of bacilli surviving in the lung one day after inhalation was detected between the control and experimental animals. In both, the ratio between the number seeded and the number recovered was close to unity. Thus, there was no diminution of the bacilli seeded in the lung one day after inhalation. In the previous AD experiments with a less virulent bacillus, the percentage survival of the bacilli in the lung at this time was definitely less.

A marked difference in the proliferation of the microorganism in the experimental and control rabbits was evident seven days after infection on direct culture. While in the control rabbits the bacillary population in the lung had multiplied 6.3 fold over the number seeded, in the experimental rabbits it had not increased at all. Similarly in the succeeding intervals, in the control rabbits, the bacilli had multiplied approximately 1,000, 26,000 and 110,000 times, respectively, on the fourteenth, twenty-first, and twenty-eighth days of infection. In the thyroxine-treated rabbits the corresponding increments were 320, 7,200, and 10,000 times over the number seeded. Thus, the inhibition of bacillary accumulation was three- to tenfold greater in the thyroxine-treated than in the simultaneously exposed control litter mates. This is clearly seen in the primitive tuberculous foci two weeks after infection in the control and experimental rabbit shown in Plate II, figures 6 and 7. The marked interstitial cellular accumulation is clearly seen in the thyroxine-treated rabbit. Essentially similar results were obtained from cultures of treated lung. The differences are clearly illustrated in text figure 5.

Special attention is called to the fact that the general form of the growth curves in the lung is the same at all times in rabbits of whatever resistance exposed to the inhalation of human tubercle bacilli. There is a lag phase, a phase of logarithmic growth, and a phase of decline which begins at the end of three weeks. This was not seen above in the AD experiment because no cultures were made in the third week. The striking similarity between the differences in the growth curves of natively resistant and natively susceptible rabbits, on the one hand, and the difference between the growth curves in the rabbits of the same genetic resistance (one group of which was under the influence of thyroxine or L-triiodothyronine and the other was untreated) is indeed of great significance.

It was found above that triiodothyronine treatment enhanced the spread of the bacilli from the lungs to the draining lymph nodes. An endeavor was made to determine whether thyroxine also affected this process. In table 9 the pertinent observations are listed. It will be noted that one day after inhalation the tracheobronchial nodes and spleens of all control and experimental animals were sterile. One week after infection these tissues were still free of tubercle bacilli in the untreated rabbits. In the thyroxine-treated animals, however, the bacilli had been carried beyond the lungs in 2 of the 3 rabbits tested: in one, to the draining lymph nodes; in the other, to the spleen. It must be emphasized that at this time about 6,000 bacilli were isolated from the lungs of the thyroxine-treated rabbits, whereas from the control litter mates, 6,000 to 110,000 microorganisms were



TEXT FIG. 5. The effect of L-thyroxine on population changes of human bacilli (H37Rv) in the lungs of III IIIC rabbits at different intervals after quantitative air-borne infection.

* Average of two determinations

TABLE 9

THE ACCUMULATION OF TUBERCLE BACILLI IN THE TRACHEOBRONCHIAL LYMPH NODES AND IN THE SPLEEN OF III IIIC UNTREATED AND THYROXINE-TREATED RABBITS AT SUCCESSIVE INTERVALS FOLLOWING THE QUANTITATIVE INHALATION OF H37Rv (STEENKEN)

Experiment of November 18, 20, and 26, 1957

Days After Infection	Rabbit Number		Number of Tubercle Bacilli in 1 gm. of Lung		Tracheobronchial Lymph Nodes				Spleen			
					Number of Tubercle Bacilli in 100 mg.		Number of Tubercle Bacilli in 100 mg. of Nodes as Per Cent of Tubercle Bacilli in 1 gm. of Lung		Number of Tubercle Bacilli		Weight in mg. per 100 gm.	
	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal
1	3-33	4-8	100	250	0	0	0	0	0	0	26.7	52.2
	3-48	3-46	825	408	0	0	0	0	0	0	50.4	64.0
	4-9	4-5	855	591	0	0	0	0	0	0	37.1	57.1
	Average		593	416	0	0	0	0	0	0		
7	3-34	3-30	658	—	0	0	0	0	0	0	32.3	40.8
	3-45	3-44	1,267	600	0	0	0	0	0	19	40.6	59.5
	4-3	4-1	10,483	467	0	5	0	1.0	0	0	34.2	63.7
	Average		4,136	534	0	2	0	0.3	0	6		
14	4-4	4-2	80,000	6,667	567	0	0.7	0	0	0	50.6	52.8
	3-36	3-35	160,000	333,634	7,867	19,000	4.9	5.6	0	0	36.7	52.5
	4-11	4-10	1,860,000	100,000	64,000	17,133	3.4	17.1	2,644	0	35.4	51.8
	Average		700,000	146,767	24,145	12,044	3.0	7.6	881	0		
21	3-32	3-31	2,370,000	697,500	114,933	7,833	4 9	1.1	0	116	37.5	54.3
	3-38	3-37	6,900,000	3,000,000	78,800	90,300	1.1	3.0	21	51	44.8	59.8
	4-7	4-6	7,133,852	3,233,000	514,667	135,000	7.2	4.2	287	1,365	29.1	47.8
	Average		5,467,931	2,310,167	236,130	77,711	4.4	2.8	103	511		
28	3-41	3-40	13,200,000	1,633,211	46,300	34,596	0.4	2.1	0	0	67.9	52.3
	3-43	3-42	6,166,382	836,528	147,667	1,971	2.4	0.2	27	0	56.3	55.9
	3-49	3-47	2,333,333	833,280	750	633	0.03	0.08	0	0	42.7	74.6
	Average		7,233,238	1,101,006	64,906	12,400	0.9	0.8	9	0	41.5 ±4.2*	55.9 ±2.03*

* These averages refer to all of the rabbits in the control and experimental groups, respectively. P value of the difference between these observations = 0.005.

cultured from the lungs. Yet their tracheobronchial nodes and spleens were sterile. In the second week after infection, the proportion of bacilli from the lung which had spread to the draining lymph node was greater in the thyroxine-treated than in the control litter mates in 2 of 3 instances and less in one. After this period there was no consistent difference in this respect between the two groups. Perhaps the larger number of bacilli recovered from the spleen of each of the 3 thyroxine-treated rabbits three weeks after infection would also suggest that the tendency for the bacilli to invade extrapulmonary tissues was greater in the experimental animals. Furthermore, this interpretation is strengthened by the fact that four weeks after infection the tracheobronchial nodes of the experimental rabbits at times contained numerous minute epithelioid cell collections without any visible bacilli, whereas in control rabbits these foci were fewer but much more extensive, containing bacilli and even caseation. This would indicate that more bacilli had colonized the nodes of the experimental rabbits but had not survived, while fewer bacilli had invaded the nodes of the

TABLE 10

THE EFFECT OF L-THYROXINE ON THE PATHOGENESIS OF TUBERCULOSIS IN III IIIC LITTER MATES TWENTY-EIGHT DAYS AFTER THE QUANTITATIVE INHALATION OF H37Rv (STEENKEN)

Rabbit Number	CC. of O_2 Consumed per Kg. 3/4 per minute	Maximal OT Sensitivity in mm.[2]	No. of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Size of Tubercle	Extent of Caseation
Control Animals					
3-41	43.4	389	12	4.0	Extensive
3-43	43.3	260	11	2.9	Moderate to extensive
3-49	46.0	414	42	3.5	Moderate, discrete
Average	44.2	354.3	21.7	3.5	
L-Thyroxine-treated Animals					
3-40	59.3*	114	41	2.5	Slight
3-42	71.0*	41	93	3.3	Slight
3-47	61.0*	280	88	3.2	Slight
Average	63.8	145.0	74.0	3.0	

* Average of O_2 consumption before exposure and 26 to 29 days after infection.

control animals but had multiplied. This is illustrated in Plate III, figures 8 and 9.

It is apparent, therefore, that in the first two to three weeks after infection there was a tendency for a greater spread of tubercle bacilli from the lungs to extrapulmonary tissues in the thyroxine-treated rabbits. However, it must be emphasized that this effect was much more conspicuous with triiodothyronine in AD rabbits than with thyroxine in the III IIIC race, and was paralleled by the much greater enhancement of resistance in the former.

It may also be noted in table 9 that there is a definite tendency for splenic hypertrophy in the thyroxine-treated as compared with the control rabbits. The spleen of the former weighed 55.9 plus-minus 2.0 mg. per 100 mg. of body weight, while that of the latter was 41.5 plus-minus 4.7. While this constitutes an hypertrophy of about 35 per cent, it must be noted that only 22 per cent of this value can be considered valid, for the thyroxine-treated rabbits weighed 12 per cent less than the controls at the time of death when their splenic weights were determined.

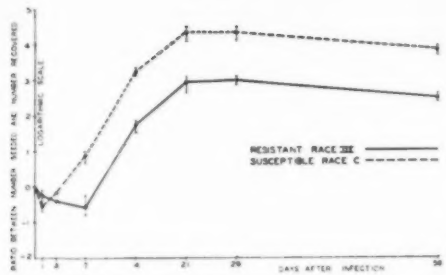
SUPPRESSION OF TUBERCULOSIS BY L-THYROXINE IN III IIIC RABBITS

The extent and character of tuberculosis developed by the III IIIC rabbits with and without thyroxine treatment twenty-eight days after infection are shown in table 10. The relative inhibitory capacities of these rabbits against the accumulation of tubercle bacilli in their lungs has just been described. The native resistance of the 3 thyroxine-treated animals was increased two to seven times over that of their control litter mates. Again, the tubercles were smaller with less extensive caseation and an accompanying reduction in the tuberculin sensitivity. In Plate III, figure 10, are illustrated the gross differences in the extent of the tuberculosis in the single lung of each of the 3 control and the 3 thyroxine-treated rabbits. The other lung of each of these rabbits was quantitatively cultured and formed the basis for the data recorded above in table 7.

DISCUSSION

It has been demonstrated that, by the administration of L-triiodothyronine or L-thyroxine for two to four weeks prior to the quantitative inhalation of virulent human tubercle bacilli and by the continued treatment with these hormones during the course of the infection, the inception and progress of the ensuing tuberculosis can be suppressed to a greater or lesser degree in the following rabbit races: AD, III IIIC, IIIA, and CaC. The increase in native resistance was as much as twenty fold in AD rabbits under the influence of triiodothyronine (*see* Plate I, figure 1) and fourfold in IIIA rabbits (*see* Plate I, figure 3) under the effects of thyroxine. The quantitative estimate of increased native resistance was based on the well-founded and constant observation that the greater the native resistance of a given race to tuberculosis, the fewer are the tubercles produced by a given number of inhaled human tubercle bacilli of a given virulence.

The enhancement of resistance produced by this treatment in the experimental rabbits was associated with an hyperthyroidism, evidenced by an average increase in basal metabolic rate of 30 per cent or more over their own base line or over the basal metabolic rate of the controls, not treated with hormones. This increased metab-



TEXT FIG. 6. Population changes of human bacilli (H37Rv) in the lungs of natively resistant and susceptible rabbits at different intervals after quantitative air-borne infection (direct culture).

olism characterized the experimental rabbits both before infection and during the course of the disease. Not only was the number of tubercles produced in the lung greatly reduced in the treated rabbits, but the progression of those formed was significantly retarded. This was apparent from the smaller size of the tubercles, their less extensive caseation, and the marked reduction in the number of bacilli contained in the lesions of the treated as compared with those of the control rabbits.

By cultural methods it was demonstrated that these hormones increased resistance by augmenting the inhibition of bacillary accumulation in the lungs. This inhibition was ten- to forty fold greater in rabbits under the influence of triiodothyronine and three to ten times greater in thyroxine-treated animals as compared to that of their respective controls.

It is noteworthy that untreated natively resistant race III rabbits differ from untreated natively susceptible race C rabbits by an innate twenty- to thirty fold greater inhibitory capacity against the accumulation of the bacilli in the lungs of the former race. Therefore, hyperthyroidism increases resistance by bacteriologic and pathologic effects which are similar to those characteristic of high native resistance. To illustrate this observation, growth curves of tubercle bacilli in the lungs of races III and C from a previous study (3) (text figure 6) are here reproduced for comparison with text figure 5 shown above. The similarity of the effect of native resistance, on the one hand, and thyroid hormone treatment, on the other, on the bacillary population changes in the lung is striking.

Moreover, it was found in the study cited above (3), contrary to expectation and the generally implied opinion of investigators, that the spread of bacilli from the lung to the draining tracheobronchial lymph nodes was greater in natively resistant than in natively susceptible rabbits. Thyroid hormones also appeared to augment the transfer of bacilli from the lung to the nodes. This interpretation is based on the assumption that bacilli present in the tracheobronchial nodes early in the course of this respiratory infection were derived not from the general circulation but directly from the bacilli carried to these nodes by the lymphatics from the lungs. This assumption is in accord with one of the most well-founded facts in the pathogenesis of tuberculosis; namely, the lymph nodes draining the portal of entry are always the first invaded by the microorganism. Again, the presence of a greater proportion of bacilli of pulmonary origin in the nodes of thyroid-treated animals cannot be attributed to a more rapid multiplication of the invading bacilli. The contrary is the case; the bacillary growth is inhibited by these hormones (*see* Plate III, figures 8 and 9). Therefore, it may be tentatively concluded that thyroid hormone treatment, like native resistance, increases the flow of tubercle-bacilli-contaminated lymph from the lung to the draining nodes. In the case of native resistance, the increased lymph flow was attributed to the increased inflammatory response of the resistant as compared to that of the susceptible animal. No definite evidence of increased inflammatory irritability in hyperthyroidism has as yet been produced. Whether the increased respiratory and circulatory activity due to hyperthyrodism is a significant factor in this relation has not been determined. The enhancing effect of hyperthyroidism induced by triiodothyronine on the spread of bacilli from the lung to the nodes was much greater than that of hyperthyroidism caused by thyroxine. The increment in bacillary spread paralleled the increment of resistance and not the increment in basal metabolic rate. This problem will be discussed further in a subsequent paper (4*b*).

Whether the increased removal of bacilli from the lungs of the hormone-treated rabbits plays a considerable role in the suppression of the bacterial population in this organ seems doubtful. This was particularly evident in the first week after infection. At this time the number of bacilli found in the extrapulmonary tissue of the hor-

mone-treated rabbits was negligible by comparison with the large numbers of microorganisms recovered from their lungs. Yet the bacillary population in the hormone-treated rabbits was suppressed six- to tenfold by comparison with that of the untreated controls.

It must be emphasized that hyperthyroidism, as here defined, does not always significantly increase resistance. This was not effected in the very susceptible race C nor, in a limited experiment, in the highly resistant race III. Moreover, triiodothyronine in the concentration present in the treated rabbits had no effect on the growth of the bacilli *in vitro*. Therefore, it is not the added hormone as such that increases resistance but rather the unknown internal changes induced by the hormone in certain races that is responsible for the observed difference.

The thyroid hormones modified the progress of the lesions in the following respects: The tubercles were much smaller (*see* Plate I, figure 3); their caseation was regularly and markedly suppressed and was often further advanced; the interstitial inflammation was accentuated; tubercle bacilli were scanty, short, and poorly stained; skeins and cords of bacilli were extremely rare by comparison with their great frequency in the controls (*see* Plate IV, figures 11 and 12); finally the resolution of tubercles by infiltration of capillaries and erythrocytes was frequently more advanced in the hormone-treated rabbits (*see* Plate IV, figures 13 and 14). All of these differences are essentially expressions of the restriction imposed by the thyroid hormones on the growth of the bacilli in the tissues.

Not only can the inception and progress of tuberculosis be markedly suppressed in certain races by thyroid hormone treatment given before challenge and during the infection, but the progress of an already existing disease of twenty-one days' duration can also be considerably checked by intensive triiodothyronine treatment; so that one-half to two-thirds of the tubercles initiated fail to attain macroscopic size when these rabbits, together with their untreated controls, are killed some six weeks later. This may be attributed to the resolution of some incipient tubercles by the increased inhibition of bacillary multiplication afforded by the hormone (*see* Plate I, figure 2).

When the multiplication of the bacilli was markedly suppressed by the hormones and when the resulting tuberculous lesions were conspicuously reduced, the tuberculin sensitivity was also sharply diminished. Mere hyperthyroidism did not diminish the allergic response. Further consideration of the effect of thyroid hormones on tuberculin sensitivity is reserved for the succeeding paper (*4b*) in which the influence of hypothyroidism on native resistance is presented.

Summary

In the studies in this laboratory on constitutional factors which may control native resistance of inbred rabbits to tuberculosis, it was found that hyperthyroidism induced by L-triiodothyronine or L-thyroxine suppressed to a greater or lesser degree the inception and progress of the pulmonary tuberculosis produced by the quantitative inhalation of human tubercle bacilli in four races, AD, III IIIC, CaC, and IIIA, with low or intermediate genetic resistance to the disease. L-Triiodothyronine also exerted a definite suppressive influence on the development of an already existing tuberculosis when the hormone was administered three weeks after the infection. The resistance of the most susceptible strain in the colony, C, could not be materially increased by this treatment.

By cultural methods it was found that the administration of both triiodothyronine and thyroxine markedly restrained the accumulation of bacilli in the lungs and increased, to a varying degree, their spread to the draining lymph nodes. Histologic studies substantiated the gross and cultural observations. Whenever these hormones exerted a conspicuous inhibitory effect on the inception and progress of the disease, there was a concomitant sharp reduction in tuberculin sensitivity. The difference in host-parasite relationships between treated and untreated rabbits of the same genetic resistance was of the same bacteriologic and pathologic nature as that between untreated natively resistant and susceptible rabbits. Genetic resistance is characterized by high innate ability of the tissues to inhibit the accumulation of tubercle bacilli and by a marked spread of the bacilli from the portal of entry to the draining lymph nodes. The administration of thyroid hormones to certain races is likewise characterized by an increased inhibitory capacity of their tissues against the accumulation of tubercle bacilli, by an enhancement of the spread of the bacilli from the lung to the draining nodes, and by an increased resistance to the disease.

Sumario

Del Papel del Tiroides en la Resistencia Natural a la Tuberculosis. I. El Efecto del Hipertiroidismo

En estos estudios de los factores constitucionales que pueden regir la resistencia natural de los conejos de raza pura a la tuberculosis, se descubrió que el hipertiroidismo inducido por L-triyodotironina o L-tiroxina suprimía en mayor o menor grado la incepción y evolución de la tuberculosis pulmonar producida por la inhalación cuantitativa de bacilos tuberculosos humanos en cuatro razas, AD, III IIIC, CaC y IIIA, dotadas de resistencia baja o intermedia a la enfermedad. La *L*-triyodotironina ejerció además un efecto supresor bien definido sobre la evolución de una tuberculosis preexistente al administrarse la hormona a las tres semanas de la infección. La resistencia de la cepa más susceptible de la colonia de conejos, C, no pudo aumentarse mayor cosa con este tratamiento. Con técnicas culturales se descubrió que la administración de triyodotironina a la par que de tiroxina limitaba notablemente la acumulación de bacilos en los pulmones y acrecentaba, en forma variable, su difusión a los ganglios linfáticos de drenaje. Los estudios histológicos apoyaron las observaciones macroscópicas y culturales. Siempre que estas hormonas ejercían notable efecto inhibidor sobre la incepción y evolución de la dolencia había una concomitante reducción aguda en la sensibilidad a la tuberculina. La diferencia en la relación de huésped-parásito entre los conejos tratados y los no tratados de idéntica resistencia genética era de la misma naturaleza bacteriológica e histopatológica que la existente entre los conejos resistentes y susceptibles naturalmente sin tratar. La resistencia genética se caracteriza por la capacidad innata de los tejidos para inhibir la acumulación de bacilos tuberculosos y por una destacada difusión de los bacilos desde la vía de entrada a los ganglios linfáticos de drenaje. La administración de hormonas tiroideas a ciertas razas se caracteriza igualmente por la mayor capacidad inhibidora de los tejidos de los mismos contra la acumulación de bacilos tuberculosos, por aumento de la difusión de los bacilos desde el pulmón a los ganglios de drenaje y por mayor resistencia a la enfermedad.

Resume

Du rôle de la thyroïde dans la résistance naturelle à la tuberculose. I. L'effet de l'hyperthyroïdie

Dans les recherches effectuées par les auteurs sur les facteurs constitutionnels aptes à contrôler la résistance naturelle des lapins consanguins à la tuberculose, on a découvert que l'hyperthyroïdie provoquée par la L-triiodothyronine ou la L-thyroxine inhibait à un degré plus ou moins élevé la primo-infection ou la progression de la tuberculose pulmonaire produite par l'inhalation quantitative de bacilles humains appartenant à quatre races: AD, III III C, CaC et IIIA, et possédant une résistance génétique faible ou modérée à l'infection tuberculeuse. La L-triiodothyronine exerçait également une influence inhibitrice définie sur le développement d'une tuberculose existante lorsque l'hormone était administrée trois semaines après l'inoculation. Ce traitement n'a pu accroître substantiellement la résistance de la souche la plus sensible dans notre colonie (C). Les méthodes culturales ont permis de découvrir que l'administration de triiodothyronine et de thyroxine réduisait nettement l'accumulation de bacilles dans les poumons, et augmentait, à divers degrés, leur dissémination dans les ganglions lymphatiques de drainage. Les études histologiques ont corroboré les observations macroscopiques et culturales. Quand ces hormones exerçaient une action inhibitrice apparente sur l'apparition et les progrès de la tuberculose, une nette réduction concommitante de l'allergie tuberculeuse existait également. La différence entre les rapports hôte-parasite des lapins traités et non traités, de même résistance génétique, était de la même nature bactériologique et pathologique que la différence existant entre les lapins non traités possédant une résistance naturelle et les lapins sensibles à l'infection. La résistance génétique est caractérisée par une très grande capacité innée des tissus à inhiber l'accumulation des bacilles tuberculeux, et à entraîner la dissémination marquée des bacilles du point d'infection aux ganglions lymphatiques de drainage. L'administration d'hormones thyroïdiennes à certaines races est également caractérisée par une augmentation du pouvoir inhibiteur de leurs tissus contre l'accumulation des bacilles tuberculeux, par une amplification de la dissémination des bacilles dans les ganglions lymphatiques de drainage à partir du poumon et par une résistance accrue contre la tuberculose.

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PLATES I–IV FOLLOW

PLATE I

FIG. 1. Upper row: One lung from untreated rabbits, AD3=51, AD4=11 and AD4=19. Lower row: One from AD5=43, AD5=26 and AD4=20 treated with triiodothyronine before and during the infection. The 6 rabbits were killed thirty days after the inhalation of 9,000 to 18,000 H37Rv bacilli. The contralateral lung from each of these rabbits was quantitatively cultured.

FIG. 2. Upper row: The lungs of untreated rabbits III IIIC1-56 and III IIIC2-20. Lower row: Those of siblings III IIIC1-52 and III IIIC2-21 treated with triiodothyronine, beginning twenty-one days after infection. The 4 rabbits were killed sixty-three to sixty-five days after the inhalation of 34,000 tubercle bacilli by rabbits -1-56 and -1-52 and 50,000 to 53,000 by rabbits -2-20 and -2-21, respectively.

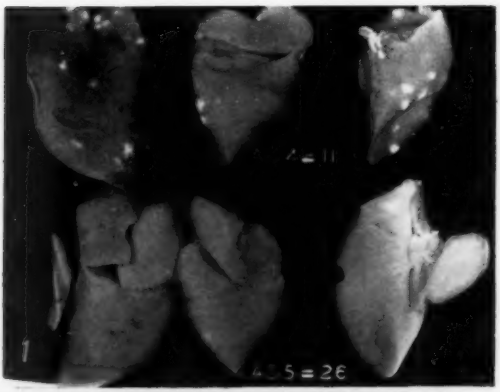
FIG. 3. The lungs of IIIA3-14, control and IIIA3-13, a thyroxine-treated sibling, thirty-four to thirty-five days after the inhalation of 9,000 and 11,000 tubercle bacilli, respectively.

AD3=51

AD4=19

AD5=43

AD4=20

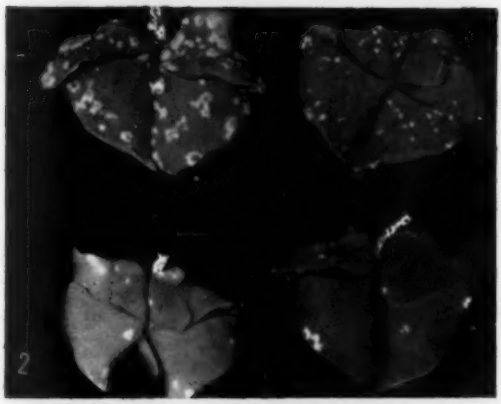


III IIIC1-56

III IIIC2-20

III IIIC1-52

III IIIC2-21



III A3-14

III A3-13

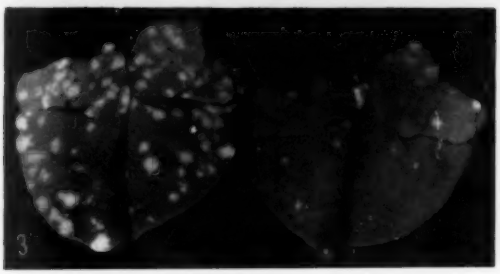


PLATE I

PLATE II

FIG. 4. Portion of a tubercle in the lung of control rabbit AD3=51, shown in Plate 1, figure 1. Extensive caseation on the left and below. Numerous well-stained tubercle bacilli within the reticulated immature epithelioid cells. The rabbit was killed thirty days after inhalation of 18,000 tubercle bacilli. (Magnification × 475)

FIG. 5. Portion of a tubercle in the lung of triiodothyronine-treated rabbit AD5=43 shown in Plate I, figure 1. Core of mature, rounded, epithelioid cells with rare poorly stained short tubercle bacillus indicated by the arrow. No caseation. Extensive perifocal infiltration with round cells. The rabbit was killed thirty days after inhalation of 19,000 tubercle bacilli. (Magnification × 475)

FIG. 6. Primitive tuberculous focus in control rabbit III IIIC4-11 two weeks after inhalation of 9,000 bacilli. Numerous tubercle bacilli within alveolar phagocytes. Some polymorphonuclears in the same alveolus. The bacilli in this lung had multiplied 2,000 fold over those seeded. (Magnification × 475)

FIG. 7. Primitive tuberculous focus in the thyroxine-treated rabbit III IIIC4-10, a sibling of III IIIC4=11 shown in figure 6, two weeks after inhalation of 18,000 tubercle bacilli. A rare bacillus within an alveolar phagocyte is indicated by the arrow. There is a marked interstitial infiltration of round cells in the neighborhood of this focus. The bacilli in this lung had multiplied 61 times over those seeded. (Magnification × 475)

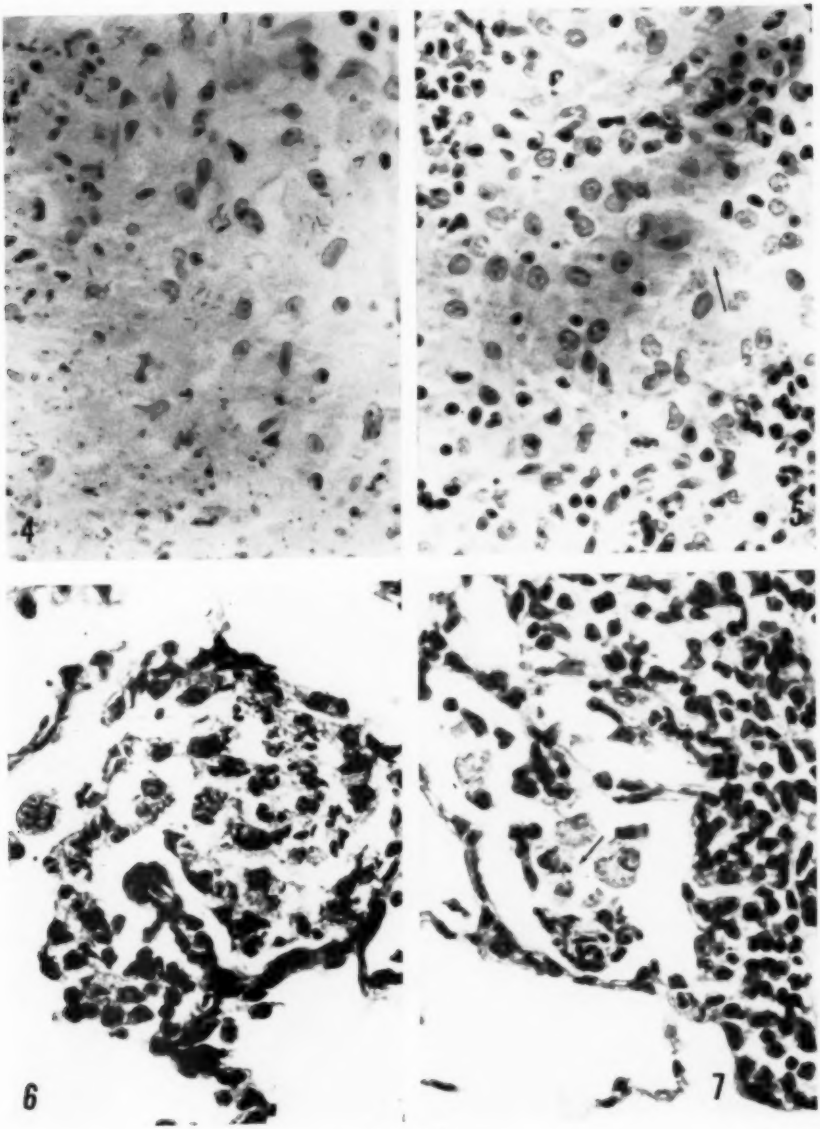


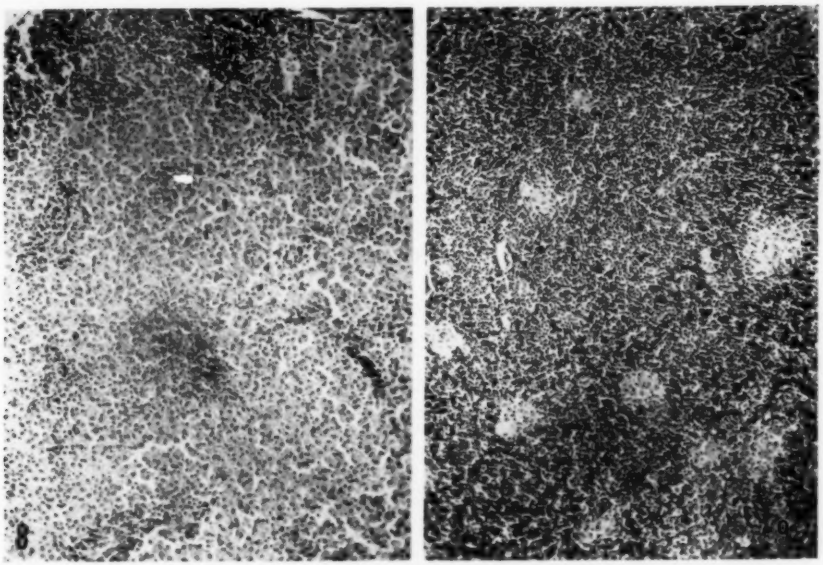
PLATE II

PLATE III

FIG. 8. Tracheobronchial node of control rabbit III IIIC3-43, twenty-eight days after inhalation of 4,500 tubercle bacilli. Extensive single tuberculous focus with caseation. Tubercle bacilli present but not visible in this photograph; 148,000 tubercle bacilli were isolated from 100 mg. of these nodes. (Magnification × 100)

FIG. 9. Tracheobronchial node of thyroxine-treated rabbit III IIIC3-42, a sibling of III IIIC3-43 shown in figure 8. Numerous minute foci of epithelioid cells. No bacilli and no caseation, indicating that many bacilli had invaded the node but were destroyed; 2,000 tubercle bacilli were isolated from 100 mg. of these nodes. (Magnification × 100)

FIG. 10. Upper row: One lung from untreated rabbits III IIIC3-41, III IIIC3-43, and III IIIC3-49. Lower row: Those from siblings III IIIC3-40, III IIIC3-42, and III IIIC3-47 treated with thyroxine before and during infection. The 6 rabbits were killed twenty-eight days after inhalation of 700 to 4,000 tubercle bacilli. The contralateral lung of each of these rabbits was quantitatively cultured.



CONTROLS

III C 3-41 III C 3-43 III C 3-49

III C 3-40 III C 3-42 III C 3-47

THYROXINE TREATED

PLATE III

PLATE IV

FIG. 11. Portion of an extensive caseous focus with much nuclear debris in a tubercle of the control rabbit, IIIA2-27. Numerous long, well-stained tubercle bacilli, some in skeins, indicated by arrows. The rabbit was killed thirty-three days after inhalation of 26,000 tubercle bacilli. (Magnification × 475)

FIG. 12. A small, amorphous, well-advanced caseous center surrounded by epithelioid cells in a tubercle of rabbit IIIA2-26, a sibling of IIIA2-27 shown in figure 11. Few poorly staining or short bacilli indicated by arrows. The rabbit was killed thirty-three days after inhalation of 29,000 tubercle bacilli. (Magnification × 475)

FIG. 13. Portion of a tubercle with extensive caseation, slight infiltration with erythrocytes, and little resolution in control rabbit IIIA3-6, thirty-three days after the inhalation of 23,000 tubercle bacilli. (Magnification × 136)

FIG. 14. The remaining core of epithelioid cells with a slight necrotic center and marked infiltration of erythrocytes, surrounded by a vast interstitial accumulation of round cells in the resolving tubercle of the thyroxine-treated rabbit IIIA3-5, a sibling of IIIA3-6 shown in figure 13. The rabbit was killed thirty-three days after the inhalation of 31,000 tubercle bacilli. (Magnification × 136)

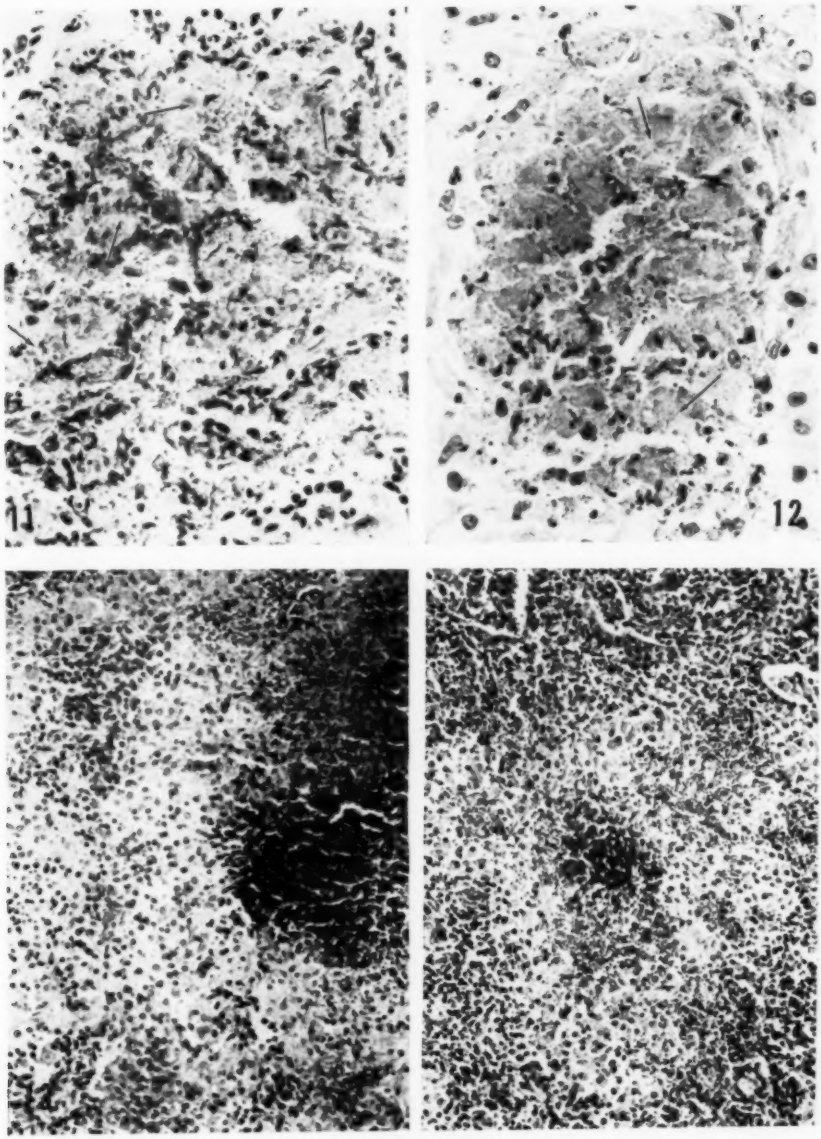


PLATE IV

ON THE ROLE OF THE THYROID IN NATIVE RESISTANCE TO TUBERCULOSIS[1,2]

II. The Effect of Hypothyroidism. The Mode of Action of Thyroid Hormones

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Introduction

In the preceding paper (1), it was demonstrated that the native resistance of some inbred rabbit races can be markedly increased by the administration of L-triiodothyronine or L-thyroxine. The resulting hyperthyroidism, as evidenced by the elevated basal metabolic rate, markedly suppressed the inception and progress of tuberculosis resulting from the quantitative inhalation of virulent human tubercle bacilli. The treated animals differed from their sibling controls of the same race by an increased inhibition of accumulation of the bacilli in the lungs and an enhanced spread of the bacilli from the lungs to the tracheobronchial nodes. Natively resistant rabbits differed from natively susceptible rabbits by the same criteria.

If hyperthyroidism increases resistance to tuberculosis, it should follow that hypothyroidism would reduce it. The purpose of this report is to demonstrate that hypothyroidism produced by thyroidectomy or propylthiouracil does indeed lower resistance. An endeavor was also made in this study to analyze, to some degree, the effects of different thyroid hormone states on the pathogenesis of tuberculosis in these inbred rabbit races.

Materials and Methods

The following races were studied: race III of very high native resistance, race IIIA of intermediate resistance, and race CaC of low native resistance. The thyroid was extirpated by blunt dissection. In no case was thyroid tissue found at autopsy in the thyroidectomized animals. In one experiment the thyroid of the litter-mate controls was exposed but not removed. The basal metabolic rate, determined as described in the preceding paper, indicated the effect of the thyroidectomy.

On a given day, in some experiments, the inflammatory irritability of the skin of the control and experimental animals was measured by injecting intracutaneously 0.03 ml. of turpentine dissolved in 0.2 ml. of sterile peanut oil. The resulting inflammation was measured two days after the introduction of the irritant. The thyroidectomized and control litter mates were then exposed to the quantitative inhalation of virulent human tubercle bacilli as in the first study. Their tuberculin sensitivity and their basal metabolic rate were also followed during the course of the infection. One or two days before the animals were killed, both experimental and control rabbits were given intravenous injections of 5 ml. of 1 per cent trypan blue in saline per kilogram of body weight in order to determine the degree of penetration of the blood-borne dye into the caseous centers of the tubercles (2).

Two strains of H37Rv were used in these experiments. For many years the virulence of H37Rv in this laboratory was constant, as determined by the number of inhaled bacilli required to generate one tubercle in a given race (3).

In March and April, 1957, there were indications that the virulence of this strain had decreased considerably. For this reason a new strain of H37Rv was obtained from Dr. William Steenken, Jr., of the Trudeau Laboratory. This "Steenken" H37Rv strain proved to be much more tuberculogenic than the old strain, so that the number of inhaled bacilli required to generate one tubercle in a given race was greatly reduced. It must be clearly understood that in any one experiment, in which the effect of hyper- or hypothyroidism was tested, both the experimental and control animals were simultaneously exposed to the same aerosol of the same strain of bacilli.

Experimental Observations

The effect of thyroidectomy in IIIA rabbits

A group of 18 litter mates of the IIIA family was selected. Nine of these animals were thyroidectomized. Two and a half to three months later the basal metabolic rate of the thyroidectomized rabbits was determined. As seen in

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Aided by grant No. E311 (C5) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service; and by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and the Westmoreland Public Health Association.

TABLE 1

THE EFFECT OF THYROIDECTOMY ON THE INCEPTION AND PROGRESS OF TUBERCULOSIS PRODUCED BY THE INHALATION OF H37Rv IN RACE IIIA RABBITS

Experiment of November 6–7, 1957

Rabbit Number*	Cc. of O_2 Consumed per Minute per kg. 3/4	Turpentine Inflammation in mm.²	Maximal OT Sensitivity in mm.²	Weight of Spleen in mg. per 100 gm.	Weight of Pituitary in mg. per 100 gm.	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Average Diameter of Tubercles	Per Cent Incidence of White Caseous Centers
Control Animals								
3-30 (a)	40.5	3,443	130	60.8	0.9	29	4.9	22
3-33 (a)	39.3	2,016	113	45.8	0.9	17	2.9	0
3-37 (a)	35.7	2,886	216	53.8	0.8	33	3.3	6
2-9 (b)	47.2	1,440	212	30.4	1.0	34	2.6	6
2-8 (b)	38.0	4,165	1,011	55.0	1.2	32	3.5	10
2-7 (b)	40.6	4,331	354	46.5	0.8	17	3.2	8
4-2 (c)	49.0	2,688	154	29.8	0.9	22	2.3	3
3-34 (d)	44.6	3,412	378	47.1	0.8	23	2.5	4
3-20 (d)	42.3	3,069	189	45.5	0.7	27	2.2	6
Mean	41.9	3,050 ± 312	306 ± 103	46.1 ± 3.5	0.9 ± 0.02	26 ± 2.2	3.0 ± 0.3	7.2 ± 2
Thyroidectomized Animals								
3-26 (a)	37.1	1,232	34	39.3	1.1	12	3.2	13
3-32 (a)	27.7	1,386	25	26.5	0.8	12	3.1	—
3-39 (a)	28.7	2,100	?	39.7	1.4	26	3.5	15
3-23 (a)	*40.8*	2,156	33	32.0	1.3	*39*	2.9	32
2-19 (b)	33.1	1,034	77	26.6	1.3	18	3.7	27
4-3 (c)	36.8	1,232	86	21.3	1.2	14	3.1	55
4-1 (c)	35.8	1,764	51	13.1	1.7	5	3.6	13
3-21 (d)	27.8	1,001	82	24.1	1.1	11	3.3	54
3-27 (e)	34.0	2,052	47	51.8	1.1	11	3.8	23
Mean.....	33.5	1,551±157	54±8.5	30.5±3.9	1.2±0.08	16±3.4	3.4±0.1	29±6
Statistical evaluation	P = <0.001	P = 0.01	P = 0.004	P = 0.001	P = 0.01	P = 0.11	P = 0.002	

* Rabbits with the same letter are litter mates.
? Anaphylactic type of reaction.

table 1, thyroidectomy reduced the oxygen consumption by about 20 per cent. At this time all of the rabbits were given 0.03 ml. of turpentine intracutaneously. The third column lists the volume of inflammation two days after the injection. It is clear that thyroidectomy reduced the inflammatory irritability of the skin to one half that of the intact litter mates. This difference is statistically highly significant. The rabbits were then exposed simultaneously to the quantitative inhalation of H37Rv "Steenken" strain.

During the course of the infection it was evident that the tuberculin sensitivity was sharply reduced in the thyroidectomized litter mates; yet the inception and progress of their disease were definitely increased. While the intact rabbits required 26 inhaled tubercle bacilli to generate one tubercle, 16 microorganisms (or 38 per cent less) sufficed for the thyroidectomized animals. This reduction in resistance was statistically significant. It may be seen that one thyroidectomized rabbit, IIIA3-23, exhibited a high resistance. It is interesting to note, therefore, that the oxygen consumption of this rabbit was not reduced by the operation. It must be emphasized that the basal metabolic rate of the control and experimental rabbits at the time of exposure to the inhalation of tubercle bacilli was taken into consideration in estimating the number of bacilli inhaled by any one rabbit. As a result, the thyroidectomized rabbits of the

present experiment, because of the 20 per cent reduction of their basal metabolic rate, inhaled fewer tubercle bacilli than their intact controls; yet, the total number of tubercles formed was distinctly greater in the experimental animals. For economy of space these figures were omitted from the table, as they enter into the standard numerical estimate of resistance given in column 7, i.e., the number of inhaled bacilli yielding one tubercle.

There was no constant difference in the size of the tubercles. However, the extent of the caseation was very much greater in the thyroidectomized than in the control litter mates. This is indicated in the last column of table 1. Here it may be seen that, whereas only 7 per cent of the tubercles in the control rabbits had confluent caseous centers so large[3] that the trypan blue failed to penetrate them—and thus appeared white—29 per cent of the tubercles in the thyroidectomized animals had white caseous centers containing very large numbers of bacilli. It is worthy of note that thyroidectomy is constantly associated with a marked atrophy of the spleen. This is an effect opposite to that of hyperthyroidism which, as is well known, causes hyperplasia of lymphatic tissue. The pituitary of the thyroidectomized rabbits was definitely increased in weight. Hyperthyroidism had no effect on the size of the pituitary.

Thus thyroidectomy enhanced the inception and progress of the disease in IIIA rabbits and was associated with an increase in the number of bacilli in the lesions and the extent of their caseation. The effect of thyroidectomy was therefore diametrically opposite to that of the administration of added thyroid hormone in this race, as recorded in the preceding paper (1).

THE EFFECT OF THYROIDECTOMY IN RACE III

It was found in a preliminary experiment in the above study that the resistance of the highly resistant race III could not be further increased by the administration of added L-triiodothyronine. This is understandable if it is assumed that a certain level of thyroid activity is optimal for resistance and that, beyond this level, a further increment of thyroid function does not increase resistance. It was reasoned that, if the thyroid plays a significant role in resistance, the elimination of the thyroid altogether should reduce resistance even in this race.

Accordingly, a group of 22 rabbits, chiefly of the sixth and seventh inbred generations of race III, was divided into control and experimental animals. Nine of the 11 pairs were of the same parentage, the other 2 were closely related genetically, although not identical. The thyroid of the experimental group was extirpated. The thyroid of the control group was exposed but not removed. Three to four months after thyroidectomy the basal metabolic rate of both groups was determined as in the experiment with the IIIA rabbits; the average reduction of the basal metabolic rate of the experimental animals was again approximately 20 per cent (table 2). At this time all rabbits, in groups of 6 (3 experimental and 3 control animals), were simultaneously exposed to the quantitative inhalation of H37Rv, the old strain from this laboratory.

During the course of the experiment it became apparent that neither the control nor the experimental animals developed constant and significant tuberculin sensitivity. Furthermore, cultures of the infected air respired by these rabbits indicated that the number of bacilli to which they were exposed was, in a considerable proportion of the control and experimental animals, below the critical number required to generate one tubercle in the rabbits of this race (*see* column 5, "Number of tubercle bacilli inhaled on first exposure"). It will be remembered that an average of about 1,000 inhaled bacilli of this strain of H37Rv had been required to generate one tubercle in 71 rabbits of this race (3). Therefore, forty-two days after the first infection, these rabbits were simultaneously exposed to a second inhalation infection of a twenty fold larger dose of the same microorganism. Needless to say, the basal metabolic rate of both groups was again determined within several days of the second exposure.

As will be noted in table 2, both the primary and secondary infections, respectively, of each rabbit (control or thyroidectomized animals) were of the same magnitude. The control and thyroidectomized rabbits were then killed after the same duration of the secondary infection. It was impossible to differentiate the pulmonary lesions due to the primary infection from those due to the secondary infection. Considering the fact that 5 of the 11 rabbits in each group inhaled less than 1,000 bacilli during the first ex-

[3] An interpretation of the pathologic significance of "white" caseous center is presented in the section "Histologic Observations."

TABLE 2

THE EFFECT OF THYROIDECTOMY ON THE PROGRESS OF TUBERCULOSIS IN RACE III RABBITS AFTER A PRIMARY AND A SECONDARY QUANTITATIVE INHALATION OF HUMAN TUBERCLE BACILLI H37Rv (PHIPPS)

Interval Between Primary and Secondary Infections—42 Days

Rabbit Number*	Cc. of O_2 Consumed per kg. $^{3/4}$ per Minute	Inflammatory Irritability in mm.[3]	Weight of Spleen in mg. per 100 gm.	Number of Tubercle Bacilli Inhaled on First Exposure	Number of Tubercle Bacilli Inhaled on Second Exposure	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Diameter of Tubercle	Per Cent of Tubercles Excavated or Liquefied
Sham-Operated Animals								
7=4 (a)	40.2	2,255	28.7	2,041	36,628	2,975	3.4	8
7=51 (b)	41.1	3,900	28.7	1,931	41,463	3,338	2.0	8
5=83 (c)	36.7	4,200	18.7	1,488	35,042	6,089	4.6	16
7=37 (d)	42.9	3,477	29.7	2,783	10,649	6,716	2.3	0
7=38 (d)	36.5	2,240	27.0	2,293	9,058	1,892	2.3	0
6=75 (f)	43.7	1,900	32.7	2,685	10,465	13,150	1.0	0
7=9 (g)	39.5	3,500	36.2	625	45,179	2,863	2.6	19
7=31 (h)	43.1	4,800	33.3	608	46,024	4,239	1.7	0
7=32 (h)	40.7	3,520	44.1	608	42,331	1,162	2.5	0
6=60 (f)	43.8	3,575	21.2	856	25,568	2,402	1.8	18
6=79(f)	42.3	3,582	29.5	784	21,253	11,019	2.8	0
Mean.....	41.0±0.8	3,359±267	30.0±2	1,500	29,400	5,100±1,200	2.5±0.3	6±2.4
Thyroidectomized Animals								
7=3 (a)	33.4	1,461	18.6	1,328	38,253	3,045	3.2	23
7=42 (b)	29.9	2,381	21.4	1,525	33,438	2,185	2.8	26
5=70 (e)	32.0	336	28.2†	1,574	33,170	1,511	3.5	4
7=53 (d)	35.5	4,412	20.7	2,587	7,466	529	5.7	90
7=43 (e)	30.9	1,936	18.0	2,117	6,928	210	2.8	9
6=59 (f)	33.1	1,019	18.5	2,352	8,874	1,871	3.3	33
6=22 (i)	35.3	1,045	23.6	621	40,626	655	3.1	8
7=44 (e)	34.0	1,360	19.0	540	39,206	764	2.8	6
7=56 (d)	32.2	3,713	17.1	554	38,354	1,768	3.1	45
6=69 (f)	30.0	2,925	12.5	672	20,934	1,271	2.2	16
6=70 (f)	35.8	3,089	16.0	745	22,532	2,328	2.0	0
Mean.....	32.9±0.6	2,152±381	18.5±1	1,300	26,300	1,500±260	3.1±0.3	24±7.8
Statistical evaluation	P = <0.001	P = 0.009	P = <0.001			P = 0.004	P = 0.07	P = 0.02

* Rabbits with same letter are litter mates.

† Carcinoma of uterus. Value for spleen excluded from mean.

posure and, furthermore, that only 4 of the 11 control rabbits reacted positively to tuberculin during the course of the first infection, it is quite likely that some of the control rabbits failed to develop any gross tubercles from the first infection. In any event, the numerical estimate of the resistance of these rabbits is based on the sum of the bacilli inhaled by each rabbit on his first and second infections combined.

It will be noted in column 7 of table 2 that an average of 5,100 inhaled bacilli was required to produce one tubercle in the control rabbits. Only 1,500 such organisms sufficed for one tubercle in the thyroidectomized animals. The resistance of this race has thus been reduced threefold by thyroidectomy. This difference is statistically significant. While the tubercles in the thyroidectomized rabbits tended to be larger than those in the experimental animals, this was not uniformly the case. However, it will be noted in the

last column of this table that the progression of the disease, as indicated by the liquefaction and excavation of the caseous tissue, was four times more frequent in the tubercles of the thyroidectomized than in those of the control rabbits. Again this difference is significant within the 2 per cent limit (Plate 1, figure 1). These liquefied and excavating lesions harbored many more tubercle bacilli than those that had not undergone this process and, in general, the lesions in the thyroidectomized rabbits contained many more microorganisms than those of the controls.

It may be noted in column 3 of table 2 that the inflammatory irritability of the skin of the thyroidectomized tuberculous rabbits was depressed below that in control rabbits when the irritant was injected on the same day, in both groups, late in the course of the second infection (three to thirteen days before they were killed). As may be seen in column 4, thyroidectomy again caused a marked atrophy of the spleen.

Thus, it is clear that thyroidectomy in race III rabbits increases the inception of pulmonary tubercles and enhances their progress by favoring the accumulation of the bacilli within the lung. While added thyroid hormone did not further increase the resistance of this highly resistant race, thyroidectomy markedly depressed this resistance, demonstrating a definite role of the thyroid in the native resistance of this highly resistant race.

THE EFFECT OF THYROIDECTOMY AND PROPYLTHIOURACIL IN CAC RABBITS

In the preliminary report of these studies (4), it was indicated that hypothyroidism induced by propylthiouracil in CaC rabbits, of low native resistance to tuberculosis, greatly enhanced the number of bacilli within the slowly maturing epithelioid cells. Since it has been found that thyroidectomy lowers the native resistance of races III and IIIA, it was thought advisable to compare the effects of thyroidectomy, on the one hand, and the suppression of thyroid function with this goitrogen, on the other, in this same CaC race.

CaC rabbits of the third to the fifth inbred generations were assigned to three groups. The majority in each group were litter mates of those in the other two groups. One group was thyroidectomized. To another group, 100 mg. of propylthiouracil suspended in saline was given subcutaneously five times a week. The sixth subcutaneous injection, on Saturday, contained 200 mg. of the goitrogen. This treatment was continued for ten weeks. At this time, the propylthiouracil-treated rabbits, the thyroidectomized rabbits (the thyroids of which had been ablated some twenty weeks previously), as well as an untreated control group, were simultaneously exposed to the quantitative inhalation of virulent H37Rv "Steenken" strain. The pre-infection treatment of the propylthiouracil group was continued at the same intervals and in the same amounts of goitrogen throughout the course of the subsequent disease. The control and thyroidectomized rabbits received no treatment at this time.

The pertinent data are presented in table 3. The oxygen consumption of the control rabbits was taken as a standard of reference in assessing the change in basal metabolism of the thyroidectomized and propylthiouracil-treated rabbits. It will be noted that both thyroidectomy and the goitrogen reduced the basal metabolic rate by an average of approximately 20 per cent. This is the same degree of hypothyroidism as was found in thyroidectomized race IIIA and III rabbits. The extraordinary goiter produced by the propylthiouracil is indicated in the third column of table 3. The thyroid of these rabbits had enlarged threefold over those of the controls. The tuberculin sensitivity was definitely reduced in both the thyroidectomized and the goitrogen-treated animals, but to a much greater degree in the thyroidectomized than in the propylthiouracil group.

It is evident from column 6 that both thyroidectomy and propylthiouracil reduced the resistance of the CaC rabbits to one half of that of the untreated intact controls. Eighteen inhaled bacilli of this very virulent H37Rv strain produced one primary pulmonary focus in the controls while 9 sufficed for both experimental groups. The difference is statistically highly significant. In both the thyroidectomized and propylthiouracil-treated animals the pulmonary tubercles were distinctly smaller and, in both, the incidence of white caseous centers was about five times greater than in the controls.

Histologic observations: The interstitial inflammation predominated in the large compact tubercles of the control CaC rabbits. Caseation was slight to moderate in extent and consisted of multiple discrete foci of necrosis with limited numbers of bacilli. These foci were separated from one another by areas of intact tuberculous granulation tissue and stretches of surviving epithe-

TABLE 3

THE EFFECT OF THYROIDECTOMY OR PROPYLTHIOURACIL TREATMENT ON THE INCEPTION AND PROGRESS OF TUBERCULOSIS IN CaC RABBITS PRODUCED BY THE QUANTITATIVE INHALATION OF H37Rv (STEENKEN)

Experiment of June 4–6, 1957. Duration of Disease 34–36 days

Rabbit Number*	Per Cent BMR† Change over Average of Controls	Weight of Thyroid in mg. per 100 gm.	Weight of Spleen in mg. per 100 gm.	Maximal OT Sensitivity in mm.[3]	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Size of Tubercle	Per Cent with White Caseous Centers
Control Animals							
3-69 (a)		7.6	45.0	400	32	3.5	10
5-14 (b)		6.5	29.7	171	13	3.5	15
5-29 (b)		7.4	34.0	419	15	3.1	13
4-17 (c)		6.2	33.0	407	13	3.4	21
5=1 (d)		5.6	30.7	85	18	3.2	3
4-24 (e)		5.3	26.0	391	20	3.3	3
5-36 (f)		6.0	30.3	162	10	3.3	4
5-28 (b)		6.7	42.6	386	19	3.8	21
Mean....		6.4±0.3	33.9±2.4	303±48	18±2	3.4±0.1	11.3±2.7
Thyroidectomized Animals							
3-8 (a)	−29		11.8	75	11	3.3	62
5-12 (b)	−35		13.8	51	6	3.0	58
5-18 (f)	−16		12.9	89	9	2.1	60
4-13 (c)	−19		19.8	61	11	2.5	56
4-21 (e)	−30		9.1	0	7	2.5	44
4-12 (c)	+4		11.0	168	*16*	2.5	75
5-4 (b)	−31		9.2	158	6	2.7	73
Mean...	−22		11.9±1.1‡	86±23‡	9±1‡	2.7±0.2‡	61.1±1.8‡
Propylthiouracil-treated Animals							
4-27 (g)	−26	13.6	35.7	253	11	2.9	16
5-26 (b)	−20	24.8	27.4	119	8	3.2	76
5-40 (f)	−7	17.4	29.7	144	9	2.4	42
4-19 (c)	−26	13.5	34.8	158	8	3.2	41
4-26 (g)	−29	13.6	34.0	278	7	2.9	16
5-38 (f)	−36	30.5	29.1	110	8	2.5	78
3-71 (h)	+17	19.2	34.6	104	*17*	3.5	74
5-15 (b)	−18	23.9	29.0	108	7	2.9	50
5-27 (b)	−25	8.8	35.0	225	6	2.5	68
Mean....	−19	18.4±2.3‡	32.1±1.1	167±23§	9±1‡	2.9±0.1‡	51.2±8.2‡

* Rabbits with same letter are siblings.

† Basal metabolic rate.

‡ The P value of the difference between these values and that of their corresponding controls is 0.001 or less.

§ The P value of the difference between this value and that of its corresponding control is 0.01.

lioid cells with few microorganisms (Plate 1, figure 2). Such tubercles were characterized by blue centers when the animals had been given trypan blue intravenously before death (*see* Plate III, figure 8). Clearly, the dye easily penetrated the small caseous foci from the ingrowing capillaries of the surrounding perifocal inflammation.

In the thyroidectomized rabbits the caseation was massive and confluent and occupied by far

the greater part of the tubercle, leaving only a narrow zone of surviving granulation tissue at the periphery. The pneumonic phase of the inflammation predominated; surviving epithelioid cells were scanty. In the extensive caseous center, plugs of caseous epithelioid cells could be easily delineated within the slightly thickened necrotic alveolar septa. These plugs frequently swarmed with immense numbers of tubercle bacilli, often in skeins. No patent blood vessels or capillaries were seen in these caseous masses (Plate 1, figure 3). The lesions in these rabbits strongly resembled those seen in cortisone-treated rabbits similarly infected with H37Rv (5). Such caseous foci had white centers when the animals had been given an intravenous injection of trypan blue a day or two before sacrifice (Plate III, figure 9). Clearly, the dye could not penetrate the centers of these large caseous masses, for the vessels had either thrombosed or their lumina had been obliterated by tuberculous endarteritis.

The same lack of penetration of the blood into the caseous foci of cortisone-treated rabbits accounts for the white caseous centers in trypan blue-injected, steroid-treated animals (Plate III, figures 10 and 11).

It is noteworthy that, despite the far greater extent of the caseation in the hypothyroid rabbits, the tubercles were definitely smaller in them than in the controls. These again resembled the small tubercles of the cortisone-treated rabbits, with their extensive central caseation and their very limited perifocal granulation tissue (5–7). The relationship between the size of the tubercle in these two altered hormone states and the suppressed tuberculin sensitivity characteristic of both will be considered later. The lesion in the propylthiouracil-treated rabbits was similar to that seen in the thyroidectomized animals except that the process tended to be less severe than in the athyroid siblings.

It has been noted in the preceding study that hyperthyroidism is often associated with an enlargement of the spleen and an enhancement of resistance. However, while thyroidectomy and propylthiouracil both exercised the same depressing effect on resistance, thyroidectomy was constantly associated with a marked atrophy of spleen; yet, propylthiouracil caused no atrophy of this organ. This difference in splenic response between the thyroidectomized and the propylthiouracil-treated rabbits is paralleled by the much greater suppression of tuberculin sensitivity in the former, as noted above. These important differences will be considered.

ON THE MODE OF ACTION OF THYROID HORMONES ON NATIVE RESISTANCE

Basal Metabolic Rate, Hyperplasia of the Thyroid, and Resistance

With this model of infection and in this species, added thyroid hormones increase resistance to a varying and, at times, to a marked degree. No depression of resistance was seen in any of these experiments. On the other hand, thyroidectomy or suppression of thyroid function by a goitrogen definitely reduced resistance in each of the three races tested. In the first instance, added thyroid hormones increased the basal metabolic rate by 30 to 40 per cent. In the second instance, ablation of the thyroid or suppression of its hormone production was associated with a 20 per cent reduction of the basal metabolic rate. To determine whether mere increase in the basal metabolic rate affects resistance, 2,4-dinitrophenol, which shares with thyroid hormones their capacity to increase oxygen consumption but is deficient in other physiologic effects of these hormones, was tested in the following experiment.

Twenty-four litter mates, mainly of the sixth inbred generation of AD rabbits, were divided into three groups. One group served as control; a second was treated with 40 γ of L-triiodothyronine three to four times a week for four weeks. These two groups, together with a third group, were then simultaneously exposed to the quantitative inhalation of H37Rv. The control animals were not treated. The triiodothyronine treatment was continued in the same dose and schedule throughout the course of the disease. The third group was treated as follows: Immediately after infection 5 mg. of 2,4-dinitrophenol per kg. in bicarbonate solution were injected subcutaneously in the morning. The same dose of crystalline material suspended in peanut oil was administered in the evening by the same route five times a week. On the sixth day, 10 mg. of this suspension per kg. were injected. On Sundays, water bottles containing 20 mg. of 2,4-dinitrophenol were offered to these rabbits. Under these conditions the basal metabolic rate of these rabbits was elevated for at least twelve of the twenty-four hours each day. All of the rabbits were killed thirty-six to thirty-seven days after infection. There was no evidence of toxic effects of the 2,4-dinitrophenol on these rabbits.

The results of this experiment are presented in table 4. It is evident that, far from increasing

TABLE 4

THE EFFECT OF DINITROPHENOL AND L-TRIIODOTHYRONINE ON THE TUBERCULOSIS PRODUCED IN "AD" RABBITS BY THE QUANTITATIVE INHALATION OF H37Rv

Experiment of November 27, 28, 1956

Rabbit Number*	Per Cent Change in BMR† during Infection	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Average Diameter of Tubercle	Rabbit Number	Per Cent in Change BMR During Infection	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Average Diameter of Tubercle	Rabbit Number	Per Cent Change in BRM During Infection	Number of Inhaled Tubercle Bacilli Yielding 1 Tubercle *Resistance*	Average Diameter of Tubercle
Control Animals				Dinitrophenol-treated Animals				Triiodothyronine-treated Animals			
6=9 (a)	+18	91	4.2	6=10 (a)	+35	27	2.8	6=11 (a)	+53	402	3.0
6=23 (b)	+1	177	4.2	6=21 (b)	+2	42	3.2	6=15 (b)	+33	66	2.8
6=32 (b)	+7	287	3.4	6=14 (b)	+30	227	3.0	6=22 (b)	+28	309	2.6
6=13 (b)	+20	150	2.6	6=24 (b)	+9	28	3.3	6=31 (b)	+40	412	2.3
6=20 (b)	+15	141	2.2								
6=16 (c)	−7	154	2.8	6=25 (c)	+30	33	3.3				
				6=17 (c)	+38	228	3.2				
				6=18 (c)	+36	83	3.3				
6=5 (d)	+29	92	2.9	6=6 (d)	+41	71	3.2	6=7 (d)	+34	181	2.3
5=20 (e)	+8	81	3.1								
4=22 (f)	+6	220	4.8								
				6=4 (g)	+34	58	3.2	6=2 (g)	+57	268	1.7
Average	+11	155±22‡	3.3±0.3	Average	+28	89±27‡	3.2±0.2	Average	+41	273±55	2.5±0.2

* Rabbits with the same letter are litter mates.

† Basal metabolic rate.

‡ P value of the difference in "resistance" between the normal and the dinitrophenol rabbits = 0.038.
P value of the difference in "resistance" between the normal and the triiodothyronine rabbits = 0.03.
P value of the difference between the size of the tubercles in the control and the triiodothyronine rabbits = 0.02.

resistance to the disease as did triiodothyronine, dinitrophenol reduced the resistance of the AD rabbits as compared with that of the controls. Yet the basal metabolic rate of the dinitrophenol-treated rabbits was raised an average of 28 per cent above their base line. A comparison of the numerical resistance of these three groups shows that the control animals required 155 plus-minus 22 inhaled bacilli to generate one tubercle. For the dinitrophenol-treated rabbits, 89 plus-minus 27 microorganisms sufficed, whereas for the triiodothyronine-treated rabbits an average of 273 plus-minus 55 bacilli was necessary to generate one tubercle. These differences are statistically valid within the 3 to 4 per cent level. Triiodothyronine definitely reduced the size of the tubercles. Dinitrophenol, while it markedly enhanced the extent of the caseation, did not increase the size of these foci.

Histologically the differences between these three groups were sharp. The caseation was most extensive in the lesions of the dinitrophenol-treated rabbits, and contained the greatest number of long, deeply stained bacilli, frequently in skeins of parallel rods. There was a pronounced pneumonic component in these tubercles. In the triiodothyronine-treated animals, on the other hand, the caseation was slight or even absent; the bacilli were few, isolated, short and, at times, poorly stained. The interstitial inflammation was preponderant and giant cells were common in the lesions. The tuberculous foci in the control rabbits occupied an intermediate position between these two groups.

Thus, both macroscopically and microscopically, dinitrophenol, despite its calorigenic effect, lowered native resistance in a race in which triiodothyronine definitely increased resistance. It was found above in triiodothyronine-treated C rabbits that, despite the marked elevation of their basal metabolic rate, there was no corresponding significant increment in resistance. Therefore, the increase of the basal metabolic rate as such, associated with hyperthyroidism,

TABLE 5

THE EFFECT OF TUBERCULOSIS OF 33–36 DAYS' DURATION PRODUCED BY QUANTITATIVE INHALATION OF H37Rv (STEENKEN) ON THE WEIGHT OF THE THYROID IN IIIA AND CaC RABBITS

IIIA			CaC		
Rabbit Number*	Absolute Weight of Thyroid in mg.	Weight of Thyroid in mg./100 gm.	Rabbit Number*	Absolute Weight of Thyroid in mg.	Weight of Thyroid in mg./100 gm.
Tuberculous					
3-30 (a)	175	4.7	5-28 (b)	173.8	6.7
2-9 (b)	209	5.1	5-29 (b)	177.8	7.4
3-20 (d)	300	8.8	5-14 (b)	183.3	6.5
3-33 (a)	159.3	5.6	3-69 (a)	189.7	7.6
4-2 (c)	185.0	6.1	4-17 (c)	186.1	6.2
3-34 (d)	223	7.2	5=1 (d)	145.9	5.6
3-37 (a)	184	6.3	5=36 (f)	143.8	6.0
2-8 (b)	160.8	4.2	4-24 (e)	128.0	5.3
2-7 (b)	233	6.3			
Mean.....	203±15	6.0±0.47		166.1±8.2	6.4±0.29
Normal					
3-26 (a)	145.8	4.4	5-12 (b)	122.4	4.2
4-3 (c)	123.8	5.5	5-4 (b)	171.8	5.5
5-21 (d)	130.3	3.6	4-12 (c)	111.8	3.0
3-32 (a)	126.2	4.0	4-13 (c)	125.6	4.0
4-1 (f)	153.8	5.5	3-8 (a)	148.8	3.7
3-27 (c)	175.8	5.3	5-18 (f)	120.0	4.3
3-39 (a)	106.3	4.0	4-21 (e)	159.4	5.0
2-19 (b)	160.1	4.6	5-2 (d)	146.4	4.9
3-23 (a)	167.7	5.2	5-6 (b)	136.4	4.9
Mean.....	143±8	4.7±0.24		138.1±6.7	4.4±0.26
	P = 0.001	P = 0.01		P = 0.01	P = <0.001

* Rabbits with the same letters are siblings.

is not the essential factor responsible for augmenting resistance.

The basal metabolic rate of normal, intact rabbits of different native resistance did not differ to any degree. Indeed, this was remarkably constant in all strains and averaged about 40 cc. of oxygen per $kg.^{3/4}$ per minute (8). Nor did tuberculosis as such elevate the basal metabolic rate; yet it was unequivocally demonstrated that thyroidectomy or suppression of thyroid function by a goitrogen reduced resistance. This would indicate that the thyroid plays a definite role in resistance. It might therefore be expected that tuberculosis would be associated with some hypertrophy of the gland. In some races the development of tuberculosis was associated with an hypertrophy of the thyroid. This is illustrated in table 5, which records the absolute and relative weights of the thyroid of normal rabbits at the time of ablation of the gland and the weights of the thyroid of litter mates thirty-three to thirty-six days after inhalation tuberculosis. It is plain that in race IIIA tuberculosis was associated with a significant enlargement of the thyroid, both absolutely and relative to their body weight. In CaC rabbits, tuberculosis was accompanied by a similar effect on the thyroid gland. In race III rabbits, the hypertrophy of the gland in this condition was not consistent.

Thus, while there was a tendency for the thyroid gland to hypertrophy in response to this type of tuberculosis, the effect was not constant. These observations would suggest that neither the basal metabolic rate, as such, nor the mass of thyroid gland alone are decisive in resistance and that some other subtle thyroid mechanism may be involved.

TABLE 6

THE EFFECT OF HYPERTHYROIDISM ON THE TUBERCULIN SENSITIVITY OF AD AND FC RABBITS PRODUCED BY THE WEEKLY INJECTIONS OF HEAT-KILLED TUBERCLE BACILLI

Rabbit Number		Average Per Cent Change in BMR* over Base Line		Days After First Injection of Heat-killed Tubercle Bacilli											
				7		14		21		28		35		42	
				Inflammation in mm²											
Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal	Control	Experimental Animal
AD5=48	AD6=29	+14.5	+50.0	0	0	14	23	61	110	198	106	455	71	585	259
AD6=28	AD6=36	+6.2	+33.4	11	7	160	31	350	62	252	84†	580	77†	359	252
AD6=34	AD6=40	−0.7	+20.6	0	104	25	22	259	167	441	134	460	73	368	113
AD6=41	AD6=42	+17.0	+33.5	96	23	320	414	292	360	324	607‡	336	484	330	436
AD7=3	AD7=5	+10.3	+23.7	16	13	90	11	138	45	192	304	276	340	113	720
Average		+9.4	+32.2	25	29	122	100	220	149	281	247	421§	209§	351	356
FC9=22	FC8=15	+4.8	+36.7	17	0	54	50	59	88	228	228	396	210	317	312
FC9=25	FC10=5	+21.7	+26.6	0	0	14	50	80	41	176	81	123	77	189	85
FC10=3	FC10=7	−2.5	+34.0	8	0	14	43	126	33	372	116	238	100	314	296
FC10=4		+10.4		0		58		87		353		205‡		119	
FC10=6		+12.8		0		76		240		315		490		290	
Average		+9.4	+32.4	5	0	43	48	118	54	289¶	142¶	290	129	246	231

* Basal metabolic rate
† Thick hairy skin
‡ Necrosis
§ P value of the difference between these observations = 0.03
¶ P value of the difference between these observations = 0.02

Rabbits were treated with 40 gm. of triiodothyronine 6 times per week for two weeks prior to first injection of heat-killed tubercle bacilli. Thereafter the rabbits received the same dose 4 to 6 times per week.

Thyroid Hormones, Allergic Sensitivity, and Inflammatory Irritability

It was found in the preceding paper that, when thyroid hormones vigorously inhibited the onset and progress of the disease, they also markedly suppressed the tuberculin sensitivity. Mere hyperthyroidism without pronounced concomitant restriction of the growth of the bacilli did not reduce the allergic inflammation. It would seem, therefore, that the low tuberculin sensitivity in tuberculosis restrained by hyperthyroidism is due to the reduced antigenic stimulation. Further evidence in support of this view is provided by the following: To determine the effect of hyperthyroidism on the development of tuberculin sensitivity under constant antigenic stimulation, a group of AD and FC rabbits with and without intense triiodothyronine treatment were sensitized by weekly intracutaneous injections of 1 mg. of heat-killed tubercle bacilli H37Rv.

The pertinent observations are recorded in table 6. It is evident that hyperthyroidism produced by triiodothyronine did not significantly reduce the tuberculin sensitivity induced with heat-killed tubercle bacilli in AD rabbits of intermediate resistance, a race in which similar treatment markedly suppressed the inception and progress of an active disease and concomitantly reduced the tuberculin sensitivity seven fold (1). The very susceptible FC rabbits developed less sensitivity than the AD rabbits, corresponding to their lower capacity for antibody production in response to antigenic stimulation with a single protein, as will be shown. In these FC rabbits hyperthyroidism sometimes reduced tuberculin sensitivity. However, the degree of suppression was not marked and was rarely statistically significant. Therefore, it is apparent

TABLE 7

THE EFFECT OF L-TRIIODOTHYRONINE ON THE ANTIBODY RESPONSE OF RACE AD AND FC RABBITS TO PRIMARY AND SECONDARY STIMULATION WITH BOVINE SERUM ALBUMIN

Rabbit	Group	BMR* Change in Per Cent	γ of Antibody Nitrogen per ml.							
			Days After Primary Stimulation				Days After Secondary Stimulation			
			7	14	21	27–28	5	10	14–15	26
AD5=48	Control	+6	0	0	23	60	171	184	125	135
AD6=28	Control	+12	0	0	0	75	106	168	113	95
AD6=34	Control	+11	0	0	57	64	251	292	295	158
AD6=41	Control	0	0	99	96	115	762	404	453	204
AD7=3	Control	−6	0	247	291	113	809	923	871	376
AD6=44	Control	—	0	0	134	112	371	417	217	—
AD6=45	Control	—	0	0	142	78	258	227	176	—
Average....		+5	0	49.4	106.1	88.1	389.7	373.6	321.4	193.6
AD6=29	Treated	+59	0	99	97	63	968	878	710	304
AD6=36	Treated	+46	0	173	109	67	827	585	526	196
AD6=40	Treated	+47	0	240	113	64	761	526	495	281
AD6=42	Treated	+12	0	176	161	109	718	719	440	226
AD7=5	Treated	+24	0	226	123	88	929	923	600	207
AD6=43	Treated	+27	0	0	79	49	254	165	130	—
AD6=47	Treated	+47	0	0	65	41	142	220	75	—
Average....		+37	0	130.6	106.7	68.7	657	575	425.1	242.8
FC9=22	Control	+5	0	25	100	69	479	308	282	124
FC9=25	Control	−5	0	0	0	0	0	0	0	0
FC10=3	Control	—	0	105	113	77	594	528	217	149
FC10=4	Control	+8	0	0	0	0	0	0	0	0
FC10=6	Control	0	0	0	50	40	280	270	136	58
FC9=14	Control	—	0	0	0	0	0	0	0	—
FC10=13	Control	—	0	0	52	43	205	164	86	—
Average....		+2	0	18.6	45.0	32.7	222.6	181.4	103.0	66.2
FC8=15	Treated	+57	0	63	92	49	159	141	103	35
FC10=5	Treated	—	0	0	0	0	0	0	0	0
FC10=7	Treated	+22	0	0	94	61	272	170	151	93
FC9=12	Treated	+63	0	0	0	0	0	0	0	—
FC9=26	Treated	+41	0	0	0	35	0	0	0	—
FC10=8	Treated	+31	0	0	0	0	0	0	0	—
FC10=12	Treated	+5	0	trace	56	46	182	117	161	—
Average.....		+36	0	9.0	34.6	27.3	87.6	61.1	59.3	42.6

* Basal metabolic rate

that the reduced tuberculin sensitivity in tuberculosis suppressed by hyperthyroidism is probably due to the inhibition of bacillary growth.

Thyroidectomy and propylthiouracil also suppressed tuberculin sensitivity in certain races, despite the fact that both procedures reduced resistance to the disease and increased the growth of the bacilli in the tissues. Evidence was presented above that thyroidectomy reduced the nonspecific inflammatory irritability of the skin to turpentine. Propylthiouracil had a similar effect on the inflammatory response to india ink (4). It is possible that the reduced inflammatory irritability induced by thyroidectomy is to some extent responsible for the lowered tuberculin sensitivity noted in races IIIA and CaC.

Cellular Activity and Thyroid Hormones

Since antibody formation may be considered a cellular function, an attempt was made to determine whether antibody production is affected by

TABLE 8

THE EFFECT OF THYROIDECTOMY ON THE ANTIBODY RESPONSE OF RACE III RABBITS TO PRIMARY AND SECONDARY STIMULATION WITH BOVINE SERUM ALBUMIN

Rabbit	Group	BMR* Change in Per Cent	Antibody Nitrogen γ per ml.						
			Days After Primary Stimulation				Days After Secondary Stimulation		
			7	14	21	28	5	10	15
III7=46	Control	+23	0	0	98	63	295	251	149
III8=21		−3	0	55	126	52	160	131	120
III8=27		−5	0	120	140	78	237	167	148
III8=30		−12	0	64	130	54	251	296	242
IIIR7=6		+1	0	0	0	0	0	0	0
IIIR7=7		−7	0	72	102	70	159	118	94
IIIR7=14		+10	0	0	57	43	113	98	77
Average...		+1	0	47 ± 17.5	93 ± 19	51 ± 9.6	174 ± 37	150 ± 37	119
III6=74	Thyroid-ecto-mized	−28	0	0	0	0	0	0	0
III8=19		−14	0	0	0	35	35	41	39
III8=28		−32	0	0	43	35	115	109	144
IIIR7=2		−11	0	0	63	39	136	139	167
IIIR7=5		−24	0	0	47	35	55	47	57
IIIR7=8		−21	0	39	102	70	119	227	266
IIIR7=10		−21	0	0	57	43	123	98	148
Average...		−22	0	6 ± 5.5	45 ± 14	37 ± 7.7	83 ± 20	94 ± 28	117
Statistical evaluation.				P = 0.02	P = 0.03	P = 0.14	P = 0.02	P = 0.13	

* Basal metabolic rate

the thyroid state. The effect of hyperthyroidism produced by triiodothyronine treatment on the production of antibodies against bovine serum albumin was studied in the race AD of intermediate resistance to tuberculosis and in the very susceptible FC strain. The treatment schedule prior to and during immunization was similar to that outlined in detail in the preceding paper for the AD race, in which it was demonstrated that this hormone markedly suppressed the growth of the bacilli in the lungs. The concentration of serum antibody nitrogen at different intervals after a single primary and a single secondary intravenous injection of 40 and 4 mg. per kg. of body weight, respectively, of purified bovine serum albumin dissolved in saline served as a measure of the rapidity and intensity of antibody production. Standard methods as outlined by Kabat and Mayer (9) were used.

The results are presented in table 7. It may first be noted in passing that the AD rabbits of intermediate resistance to tuberculosis produced much higher antibody levels, both on primary and secondary stimulation, with this nonspecific antigen than the FC rabbits, which are very susceptible to this disease. The difference in antibody response in these two races is similar to the difference in tuberculin sensitivity developed in them when sensitized with heat-killed tubercle bacilli. Furthermore, it will be noted in columns 5, 6, and 8 of table 7 that, in general, those rabbits which reacted most markedly to the first stimulation with bovine serum albumin also reacted most intensely five days after the secondary stimulation. Thus the response to a secondary stimulation with this nonspecific antigen, like acquired resistance to tuberculosis, is superimposed on the level of the first reaction to the antigen.

In AD rabbits, hyperthyroidism tended to accelerate antibody formation on primary stimulation and to intensify the early response to secondary stimulation, although the differences were not statistically significant. By contrast, similarly induced hyperthyroidism had no effect on the primary response of FC rabbits and appeared to reduce the antibody response to secondary antigenic stimulation, if it had any effect at all.

On the other hand, by similar methods hypo-

thyroidism induced by thyroidectomy definitely and significantly retarded and depressed antibody formation on both primary and secondary stimulation of race III rabbits. This is evident from the data in table 8. It is noteworthy that thyroidectomy reduced resistance to tuberculosis in this race.

Discussion

There is an extensive literature on the role of the thyroid in resistance to tuberculosis which dates to the times of Virchow and Rokitansky. It is beyond the scope of this paper to review this large field. Suffice it to refer the reader to the comprehensive and critical review of this entire subject by E. L. Schäfer (10) in 1954. While there is still disagreement, the preponderance of available experimental evidence favors the view that, within certain limits, hyperthyroidism exerts a favorable influence on tuberculosis while hypothyroidism exercises an opposite effect.

The studies reported in this and in the preceding communication (1) are perhaps adapted to answer more precisely the questions posed. The measure of resistance was not the death of the animal but, rather, the intimate host-parasite interactions in the lesions of inbred rabbits with constant natural resistance to tuberculosis. The lesions were produced by the quantitative inhalation of human tubercle bacilli, microorganisms to which the rabbit, as a species, is relatively resistant. The lesions induced by the limited growth of the bacilli tend to regress and are accompanied by little overt toxicity. This method permits the evaluation in a quantitative manner of differences in resistance which might escape detection by more involved and coarser measurements, such as death from a fatal infection.

In summarizing the data presented in these papers, it may be said that, by inducing hyperthyroidism (as evidenced by an increase in the basal metabolic rate of 30 to 40 per cent) by the administration of L-triiodothyronine or L-thyroxine, the inception and progress of the primary pulmonary tubercles that result from the inhalation of known numbers of virulent human tubercle bacilli can be suppressed in certain rabbit races to a greater or lesser degree. This seems due primarily to a marked inhibition of the growth of the bacilli in the tissues of the hyperthyroid rabbits. Conversely, thyroidectomy or suppression of thyroid hormone secretion by propylthiouracil had the opposite effect, namely: it increased the number of tubercles generated by a given number of inhaled bacilli (*see* Plate I, figure 1); greatly augmented the number of microorganisms; and intensified the progress and caseation of the lesions (Plate II, figures 4, 5, 6, and 7).

These results, on the surface, appear to be utterly different from those of Dubos and his co-workers (11) on acute tuberculosis and staphylococcal infections in mice or those of Nutter, Gemmil, and Myrvik (12) on tuberculosis and pneumococcal infections in the same animal. However, these investigators found no evidence that the greater mortality of the mice fed certain amounts of thyroid extract or L-triiodothyronine was due to an enhancement of bacterial proliferation. Rather, Smith and Dubos (13*a*) are of the opinion that, in some nonspecific manner, thyroid extract increases the susceptibility of the mouse to toxins or other bacterial products. It has been reported that triiodothyronine significantly enhances the lethal effect of Brucella endotoxin (13*b*). Susceptible to similar interpretation are the endocrinologically well-controlled experiments of Reichlin and Glaser (14) with thyroxine and propylthiouracil on acute streptococcal pneumonia of rats, in which they found decreasing mortality rates with decreasing levels of thyroid function. Although they did not study the fate of the bacteria in the lesions, their findings agree with those of the present writers in that thyroxine did not increase the extent of the lesion of these rats; initially, hypothyroid rats showed decreased resistance to the infection. Whether these differences between the observations of the present investigators and those just cited are all due to the practical absence of toxic effects in the tuberculosis caused by human bacilli in the rabbits or, in addition, to species differences as well is not immediately apparent.

As emphasized in the first paper (1), genetic resistance is characterized by a high innate ability of the tissues to inhibit the accumulation of the bacilli and by a marked spread of the bacilli from the portal of entry to the draining lymph nodes. Similarly, both triiodothyronine and thyroxine treatment restricted bacillary multiplication and enhanced the spread of bacilli from the site of invasion. The analogy also holds for the character of the lesion, namely: interstitial inflammation is accentuated, maturation of epi-

thelioid cells and caseation are more rapid, vascularization and resorption of the tubercle are more pronounced in both the natively resistant and the hormone-treated animal.

On the other hand, thyroidectomy produces the opposite effect on the character of the lesion. It increases the number of bacilli present (*see* Plate II, figures 6 and 7), accentuates the pneumonic phase, and markedly enhances the extent of its caseation (*see* Plate II, figures 4 and 5). Propylthiouracil has a similar though less baneful influence on the character of the lesion.

The effects of thyroidectomy on the pathogenesis of tuberculosis resemble in many ways those of cortisone administration. Both increase the number of bacilli in the lesion and intensify the pneumonic component. Both depress the perifocal inflammation, the tuberculin sensitivity, and antibody production. Both impede the penetration of blood-borne substances to the caseous centers (*see* Plate III, figures 10 and 11). Finally, cortisone retards the spread of bacilli from the primary focus to the draining lymph nodes. Whether thyroidectomy has a similar effect on the spread of the bacilli is being determined.

It would appear from the above that there are certain patterns of tissue responses which characterize resistance and certain others which characterize susceptibility. Whether the resistance is due to native constitutional factors or to added thyroid hormone, the tissue responses are similar. On the other hand, whether susceptibility is due to inherent genetic factors or induced by thyroidectomy or the administration of cortisone, the pathogenetic characters are again similar. This is consistent with the hypothesis that the level of corticosteroids and of thyroid activity may play a significant role in native resistance to tuberculosis. A lowering of thyroid activity tends to diminish resistance; an increase of its activity tends to increase resistance. Significantly, tuberculosis tends to induce hyperplasia of the thyroid in certain races. However, as emphasized in the text, neither the size of the thyroid nor the basal metabolic rate is related to resistance. Whether the utilization of the hormones differs in races of different native resistance is unknown.

The significance of the enhanced spread of bacilli from the lung to the draining lymph nodes in hyperthyroid rabbits is problematical. Whether there is any relationship between rate of lymph flow and hyperplasia of lymphoid tissue seen in thyroxine-treated rabbits is not known. The increased spread cannot be completely accounted for by the increased respiration and blood flow characteristic of the hyperthyroid state. For, as pointed out in the first paper, the increment in bacillary spread parallels the increment of resistance and not the increment of basal metabolic rate. Again, this spread is greater in untreated natively resistant than in natively susceptible rabbits. Yet, their basal metabolic rate is the same.

There is no clear evidence that either hyperthyroidism or native resistance is characterized by an increased inflammatory irritability of the tissues. However, the inflammatory response to inhalation tuberculosis is greater in natively resistant rabbits (3). An alluring concept, but at present devoid of evidence, is that the increased inhibition of bacillary accumulation characteristic of both is in some way (such as the possible greater release of irritating substances from dying bacilli (15)) responsible for the increased inflammatory response and hence for the increased lymph flow and spread of bacilli from the site of invasion. Continued studies on hyperthyroid and thyroidectomized animals may shed some light on this problem.

Closely allied to the inflammatory process is the effect of thyroidectomy on the pathogenesis of tuberculosis. For not only does thyroidectomy reduce resistance and profoundly modify the character of the lesion as described above; it also suppresses inflammation and causes atrophy of the spleen. It is noteworthy that cortisone, which also lowers resistance to tuberculosis and induces lesions similar to those of thyroidectomized rabbits, is a powerful anti-inflammatory agent and is also associated with a marked atrophy of the spleen. Yet, propylthiouracil, although it reduced resistance to the inception of tuberculosis, caused no splenic atrophy, and the progress of the lesions produced in such rabbits was less severe than that in thyroidectomized animals. Furthermore, the tuberculin sensitivity, though reduced in both thyroidectomized and goitrogen-treated rabbits, was much less depressed in rabbits treated with propylthiouracil. These observations would suggest that the mode of action of these two antithyroid procedures are not identical and that the spleen may also play a role in the process.

The role of inflammation in the pathogenesis

of tuberculosis brings us to the question of the effect of thyroid states on tuberculin sensitivity. Both thyroidectomy and propylthiouracil, despite the large numbers of bacilli that accumulated in the tissues under their influence, tend to lower allergic sensitivity. Koch and Schäfer (16) found that propylthiouracil reduces fibrous tissue formation. Dziewiatkowski (17) demonstrated that thyroidectomy diminishes the synthesis of chondroitin sulfate in the skin. These observations are consistent with the anti-inflammatory effects of both antithyroid procedures observed in the present studies. Thus, the reduction of tuberculin sensitivity in the hypothyroid state may result from a similar anti-inflammatory effect as exerted by cortisone. Likewise the reduction in perifocal inflammation about the caseous centers of thyroidectomized (*see* Plate II, figures 4 and 5) and steroid-treated rabbits may be similarly understood.

On the other hand, Taubenhaus and Amromin (18) have shown that fibrous tissue formation suppressed by hypophysectomy or thyroidectomy can be partially restored by thyroxine. Therefore, thyroid hormones appear to support the inflammatory process. In the light of these observations, it is noteworthy that, when triiodothyronine or thyroxine exercise their marked suppressive effect on the multiplication of the bacilli, the tuberculin sensitivity is profoundly reduced. When the hormone exerts a minor suppressive effect on the progress of tuberculosis, the tuberculin sensitivity is not diminished. Hence, mere hyperthyroidism is not anti-allergic. Moreover, allergic sensitivity produced in rabbits by killed tubercle bacilli is not significantly affected by the hyperthyroid state. Therefore, the anti-allergic effect of hyperthyroidism observed under the conditions noted above may be attributed largely to a reduction in antigenic stimulation resulting from the suppression of bacillary growth.

To digress a bit, the present writers are well aware that the level of protein-bound iodine in the blood, the radioactive iodine uptake by the thyroid, and the rate of release of I^{131} from the thyroid would, together with the basal metabolic rate, be a more accurate index of thyroid activity in these animals than the basal metabolic rate alone. Also, with the compulsory standardization of environmental factors of these inbred rabbits, it was not possible to feed the experimental animals a low iodine ration, a condition most suitable for studies on thyroid function. Yet, it would appear that the desired hyper- or hypothyroid state was achieved in these experiments, as indicated by the respective increase or decrease in the basal metabolic rate. For, it was definitely established that mere elevation of the basal metabolic rate by 2,4-dinitrophenol decreased resistance and that, in the very susceptible C race, the administration of triiodothyronine markedly increased the basal metabolic rate but failed to increase resistance. These facts would indicate that the basal metabolic rate change observed in hormone-treated animals, the resistance of which was markedly increased by triiodothyronine and thyroxine, was only one of the indices of thyroid function. This is particularly convincing since thyroidectomy invariably lowered resistance.

What is it in the hyperthyroid state which tends to suppress bacillary multiplication, and why does thyroidectomy reduce resistance? As stated above, mere increase in metabolic rate does not increase resistance. This was demonstrated with dinitrophenol in AD rabbits, the resistance of which was markedly decreased by this calorigenic agent, while triiodothyronine definitely increased the resistance of these rabbits. It is noteworthy that dinitrophenol lowers the protein-bound iodine of the blood and suppresses the thyroid-stimulating hormone of the pituitary (19).

That hyperthyroidism may increase cellular activity was suggested by the antibody response of AD rabbits. Triiodothyronine treatment of these rabbits tended to accelerate antibody formation on primary stimulation and to intensify the early response to secondary stimulation with bovine serum albumin. However, this was not constant. Long and Shewell (20) found that thyroxine markedly increased the production of diphtheria antitoxin in guinea pigs. In the susceptible FC rabbits this treatment failed to influence antibody formation. This corresponds in some degree to the increase in resistance against tuberculosis afforded AD rabbits by hyperthyroidism and to the lack of significant increment in resistance conferred on FC rabbits by thyroxine (unpublished observations). It is perhaps worth noting here that the response to a secondary stimulation with a nonspecific antigen, like acquired resistance, is superimposed on the level of the first reaction to the antigen.

On the other hand, thyroidectomy definitely and significantly retarded and depressed antibody formation on both primary and secondary stimulation with this antigen. Whether this signifies a depression of individual cellular activity or is merely a reflection of the reduced number of antibody-producing cells, associated with the atrophy of the lymphoid tissue which constantly accompanies thyroidectomy, cannot be said. Further studies on the effect of the thyroid on the activity of the reticuloendothelial system are now in progress. This is all the more urgently needed in the light of recently accumulating evidence that bacterial endotoxins increase the activity of the reticuloendothelial system under certain conditions (21). Whether thyroid activity which is influenced by endotoxins also affects this system should be determined. These studies demonstrate the important and many-faceted role of the thyroid and other hormones among the nonspecific factors in resistance to infection.

Summary

This study on the effect of hypothyroidism on the pathogenesis of tuberculosis is complementary to the preceding paper on hyperthyroidism. Both deal with the effect of the endocrine balance on the onset and progress of individual pulmonary lesions, produced by the quantitative inhalation of human tubercle bacilli.

Hypothyroidism induced by thyroidectomy or propylthiouracil reduced the native resistance of inbred rabbits to the inception and progress of this disease. This was largely effected by an enhancement of the accumulation of the bacilli in the tissues and the consequent extension of caseation. Hyperthyroidism, on the other hand, by greatly inhibiting the growth of the bacilli enhanced resistance and restricted caseation; like native resistance, it increased the spread of the bacilli from the site of invasion to the draining nodes.

Thyroid hormone treatment tended to induce hypertrophy of the spleen; thyroidectomy, a marked atrophy; and propylthiouracil, no change.

Despite the marked accumulation of bacilli in the lesions of hypothyroid rabbits, their tuberculin sensitivity was markedly reduced, particularly in thyroidectomized rabbits. Similarly, the perifocal inflammation about the extensive caseous foci in the latter was reduced. This is ascribed to the reduced inflammatory irritability of the tissues in the hypothyroid state. In hyperthyroidism, when the growth of the bacilli was markedly inhibited, the tuberculin sensitivity was reduced many fold. This is attributed to the diminution of antigenic stimulation, for hyperthyroidism did not significantly affect tuberculin sensititivity induced by heat-killed tubercle bacilli.

The host-parasite relationship in the hypothyroid rabbits is, in many respects, similar to that of natively susceptible rabbits or rabbits under the influence of cortisone; this relationship in hyperthyroid rabbits is similar to that of natively resistant animals. These parallels are shown by the growth and destruction of bacilli, by the extent of caseation, and (to some extent) by the spread of bacilli from the site of invasion.

Little can be said of the mode of action of thyroid states on enhancing or suppressing the growth of the bacilli. Mere increase in oxygen consumption is not a factor in this process, for a calorigenic agent, dinitrophenol, reduced resistance and increased the number of bacilli in the lesions; and an elevation of the basal metabolic rate of certain races by triiodothyronine failed to increase resistance. In certain rabbits, hyperthyroidism tended to accelerate antibody formation to a nonspecific antigen, bovine serum albumin. Thyroidectomy definitely and significantly retarded and depressed antibody formation to this antigen. Whether this is due to a reduction of individual cellular activity or to other factors has not been determined.

There was no difference in the basal metabolic rate of natively resistant and susceptible rabbits; however, tuberculosis caused hypertrophy of the thyroid gland in certain untreated rabbits. The differing host-parasite relationships in different thyroid states are consistent with the hypothesis that thyroid function is one of the constitutional factors controlling native resistance to tuberculosis in rabbits.

Sumario

Del Papel del Tiroides en la Resistencia Natural a la Tuberculosis. II. El Efecto del Hipotiroidismo; El Modo de Obrar de las Hormonas Tiroideas

Este estudio del efecto del hipotiroidismo sobre la patogenia de la tuberculosis complementa el trabajo anterior sobre el hipertiroidismo. Ambos tratan del efecto del equilibrio endocrino sobre la

iniciación y evolución de las distintas lesiones pulmonares, producidas por la inhalación cuantitativa de bacilos tuberculosos humanos.

El hipotiroidismo inducido por la tiroidectomía o el propiltiouracilo rebajó la resistencia natural de conejos de raza pura a la incepción y evolución de la enfermedad. Esto se efectuó en gran parte por un aumento de la acumulación de los bacilos en los tejidos y la consiguiente extensión de la caseación. Por otro lado, el hipertiroidismo, por inhibir considerablemente la proliferación de los bacilos, realzó la resistencia y limitó la caseación; lo mismo que la resistencia natural, aumentó la difusión de los bacilos del sitio de invasión a los ganglios de drenaje.

La tirohormonoterapia tendió a inducir hipertrofia del bazo; la tiroidectomía, atrofia señalada; y el propiltiouracilo no produjo cambio alguno.

A pesar de la decidida acumulación de bacilos en las lesiones de los conejos hipotiroideos, re redujo notablemente la sensibilidad de los últimos a la tuberculina, sobre todo en los tiroidectomizados. En forma semejante, se redujo la inflamación perifocal alrededor de los extensos focos caseosos en los últimos, lo cual se imputa a la reducida irritabilidad inflamatoria de los tejidos en el estado hipotiroideo. En el hipertiroidismo, cuando se inhibió notablemente la proliferación de los bacilos, se redujo a valores muy inferiores la sensibilidad a la tuberculina. Esto se atribuye a la disminución de la excitación antigénica, dado que el hipertiroidismo no afectó mayor cosa la sensibilidad a la tuberculina inducida por bacilos tuberculosos matados al calor.

La relación de huésped-parásito en los conejos hipotiroideos es, en muchos sentidos, semejante a la de los conejos susceptibles naturalmente que se hallan bajo el influjo de la cortisona; esta relación en los conejos hipertiroideos es semejante a la de los animales resistentes naturalmente. Revelan estos paralelos la proliferación y destrucción de los bacilos, la extensión de la caseación y (hasta cierto punto) la difusión de los bacilos desde el sitio de invasión.

Cabe decir poco acerca del modo de acción de los estados tiroideos sobre la acentuación o supresión de la proliferación bacilar. El mero aumento del consumo de oxígeno no constituye un factor en este proceso, porque un agente calorígeno, el dinitrifenol, rebajó la resistencia y acrecentó el número de bacilos en las lesiones; y una elevación del coeficiente del metabolismo basal de ciertas razas con la triyodotironina no hizo subir la resistencia. En ciertos conejos, el hipertiroidismo tendió a acelerar la formación de anticuerpos a un antígeno anespecífico, la seroalbúmina de bovino. La tiroidectomía retardó deprimió neta y notablemente la formación de anticuerpos a este antígeno. No se ha determinado si esto se debe a reducción de la actividad celular del individuo o a otros factores.

No hubo la menor diferencia en el coeficiente del metabolismo basal entre los conejos resistentes y los susceptibles naturalmente; sin embargo, la tuberculosis provocó hiñertrofia del tiroides en ciertos conejos no tratados. Las diversas relaciones de huésped-parásito en los distintos estados tiroideos son compatibles con la hipótesis de que la función tiroidea es uno de los factores constitucionales que gobiernan la resistencia natural a la tuberculosis en los conejos.

Resume

Du rôle de la thyroïde dans la résistance naturelle à la tuberculose. II. L'effet de l'hypothyroïdie—Mode d'action des hormones thyroïdiennes

Cette étude de l'effet de l'hypothyroïdie sur la pathogénèse de la tuberculose représente un supplément à l'article précédent relatif à l'hyperthyroïdie. Ces deux articles traitent des effets de l'équilibre hormonal sur l'apparition et les progrès des lésions pulmonaires individuelles produites par l'inhalation quantitative de bacilles tuberculeux humains. L'hypothyroïdie provoquée par la thyroïdectomie ou le propylthiouracil a réduit la résistance innée des lapins à la primo-infection et à la progression de la tuberculose. Ce phénomène était largement influencé par l'amplification de l'accumulation de bacilles tuberculeux dans les tissus et l'extension consécutive de la caséification. L'hyperthyroïdie, au contraire, en inhibant considérablement la croissance des bacilles, augmentait la résistance et restreignait la caséification; de même que la résistance naturelle, elle augmentait la dissémination des bacilles à partir du point d'infection vers les ganglions lymphatiques de drainage.

L'hormonothérapie thyroïdienne tendait à entraîner l'hypertrophie de la rate; la thyroïdectomie une atrophie marquée; le propylthiouracil ne provoquait aucun changement.

En dépit de l'accumulation marquée des bacilles dans les lésions du lapin hypothyroïdien, la sensibilité à la tuberculine était fortement réduite, surtout chez l'animal thyroïdectomisé. Chez ce dernier, la réaction inflammatoire périfocale au niveau du foyer caséeux extensif était également réduite. Ce fait est attribué à la réduction de l'irritabilité inflammatoire des tissus dans l'état d'hypothyroïdie. Dans l'hyperthyroïdie, quand la croissance des bacilles était nettement inhibée, la sensibilité à la tuberculine était considérablement réduite. On attribue ces phénomènes à la diminution du stimulus antigénique, car l'hyperthyroïdie

n'affectait pas de façon significative la sensibilité à la tuberculine provoquée par les bacilles tuberculeux tués par la chaleur.

La relation hôte-parasite chez les lapins hypothyroïdiens est sous de nombreux aspects semblable à celle existant chez les lapins ayant une sensibilité naturelle ou chez lapins qui sont soumis à l'influence de la cortisone; cette relation chez les lapins hyperthyroïdiens est semblable à celle enregistrée chez l'animal possédant une résistance naturelle. Ces éléments parallèles sont révélés par l'accroissement de la destruction des bacilles, l'étendue de la caséification et (dans une certaine mesure) par la dissémination des bacilles à partir du foyer de primo-infection.

Il y a peu à dire sur le mode d'action des états thyroïdiens sur l'amplification ou l'inhibition de la croissance des bacilles. Le simple accroissement de la consommation d'oxygène n'intervient pas dans le processus, car un agent calorigène, le dinitrophénol, réduisait la résistance et augmentait le nombre des bacilles dans les lésions. De plus, l'élévation du taux du métabolisme basal de certaines races par la triiodothyronine n'a pas permis d'augmenter la résistance. Chez certains lapins, l'hyperthyroïdie tendait à accélérer la formation d'anticorps contre un antigène non spécifique, albumine sérique bovine. La thyroïdectomie a nettement, et de façon significative, retardé et réduit la formation d'anticorps à cet antigène. On n'a pas élucidé s'il s'agissait là d'une réduction de l'activité cellulaire individuelle ou d'autres facteurs.

Il n'y avait aucune différence dans le taux du métabolisme de base entre les lapins ayant une résistance naturelle et les lapins sensibles à l'infection. D'autre part, la tuberculose entraînait l'hypertrophie de la thyroïde chez certains lapins non traités. Les rapports hôte-parasite différents dans les différents états thyroïdiens corroborent la conception considérant la fonction thyroïdienne comme l'un des facteurs constitutionnels contrôlant la résistance naturelle à la tuberculose chez le lapin.

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PLATE I

FIG. 1. The lungs of thyroidectomized rabbit III7=53 on the left and of the sham-operated sibling, III7=38, on the right. The first rabbit inhaled 2,600 H37Rv on first exposure and 7,500 bacilli on the second exposure forty-two days later. The second rabbit inhaled 2,300 and 9,100 bacilli, respectively, at the same intervals. Both were killed sixty-one days after the second exposure. Seventeen of the 19 tubercles found in the thyroidectomized rabbit were excavated or liquefied in the center. None of the 6 tubercles in the sham-operated rabbit were so affected.

FIG. 2. Portion of a tubercle in control rabbit CaC4-24 thirty-six days after inhalation of 6,600 bacilli. There is a small caseous focus with few bacilli surrounded by intact epithelioid cells and penetrated by polymorphonuclears. Such caseous foci stain blue after intravenous injection of trypan blue (*see* Plate III, figure 8). (Magnification × 400)

FIG. 3. Portion of a vast caseous center of a tubercle in thyroidectomized rabbit CaC4-12 thirty-six days after inhalation of 3,800 tubercle bacilli. Intra-alveolar plugs of caseous tissue swarming with tubercle bacilli, some in skeins. The necrotic interalveolar septa are scarcely thickened. There are no patent blood vessels. Such caseous foci are not penetrated by intravenously injected trypan blue (*see* Plate III, figure 9). (Magnification × 400)

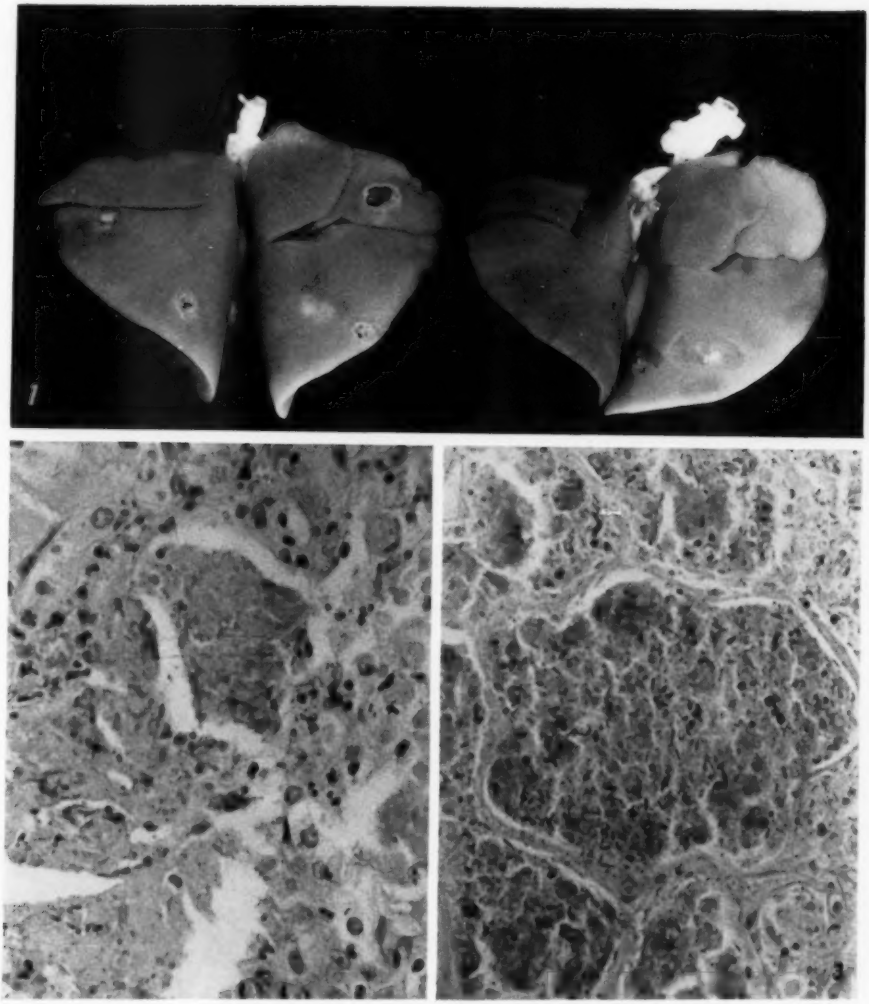

PLATE I

PLATE II

FIG. 4. Portion of a tubercle in control rabbit CaC3-69 thirty-four days after inhalation of 5,600 tubercle bacilli. Extensive interstitial perifocal inflammation about slight caseous center. (Magnification × 100)

FIG. 5. Portion of a tubercle of thyroidectomized rabbit CaC3-8, a sibling of CaC3-69 shown in figure 4, thirty-five days after inhalation of 4,600 tubercle bacilli. Vast caseous center with intra-alveolar plugs. Little perifocal, pneumonic infiltration (Magnification × 100)

FIG. 6. Portion of a tubercle of control rabbit shown in figure 4. Few tubercle bacilli in transition zone. Note capillary entering caseous center on left, above. Mature epithelioid cells without microorganisms. (Magnification × 475)

FIG. 7. Portion of a tubercle in thyroidectomized rabbit CaC3-69, adjacent to the one shown in figure 5. Numerous tubercle bacilli within the reticulated, immature epithelioid cells. (Magnification × 475)

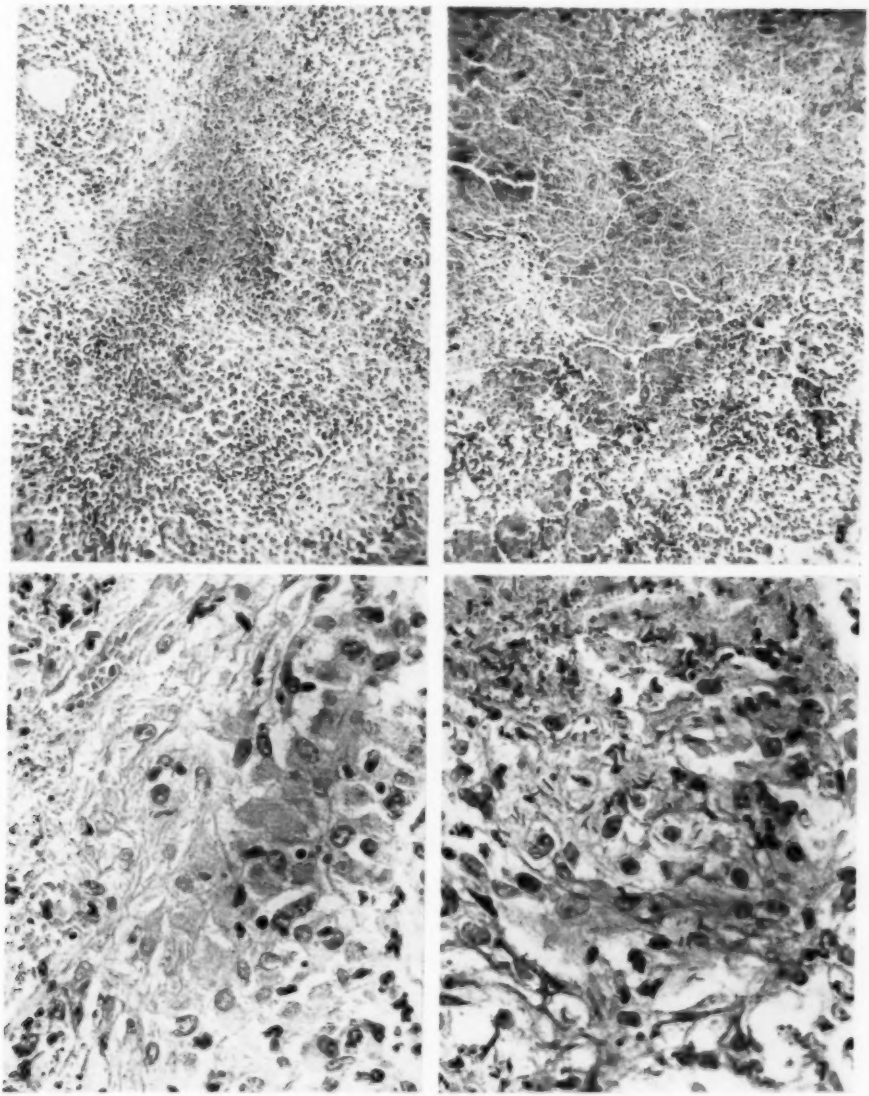

PLATE II

PLATE III

FIG. 8. The lungs of control rabbit CaC5-28 thirty-five days after inhalation of 4,900 tubercle bacilli and one day after intravenous injection of trypan blue. The tubercles are of moderate size and all but a few show blue centers.

FIG. 9. The lungs of thyroidectomized rabbit CaC5-4, a sibling of the rabbit shown in figure 8 thirty-five days after the inhalation of 3,400 bacilli and one day after intravenous injection of trypan blue. The tubercles in the thyroidectomized rabbit are smaller than those of the control. They also show white caseous centers much more frequently.

FIG. 10. The lungs of control rabbit FC7=9, thirty-six days after inhalation of 24,000 H37Rv and one day after intravenous injection of trypan blue. The caseous foci in nearly all tubercles appear as blue spots surrounded by considerable perifocal infiltration.

FIG. 11. The lungs of FC7=11, a cortisone-treated sibling of FC7=9 shown in figure 10 thirty-seven days after inhalation of 24,000 tubercle bacilli and two days after intravenous injection of trypan blue. The caseous focus of most tubercles is devoid of dye and appears white. The perifocal infiltration in the cortisone-treated rabbit is less, and the tubercles are usually smaller than in the control rabbit.

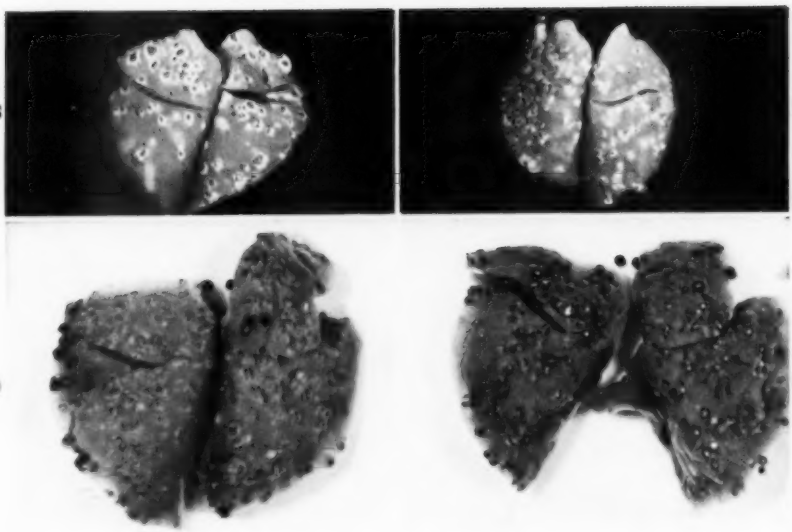

Fig. 8 Fig. 9

Fig.10 Fig.11

PLATE III

THE INDICATIONS AND USE OF PRE-RESECTION AND POST-RESECTION THORACOPLASTY IN TUBERCULOSIS[1,2]

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Introduction

Resectional surgery for pulmonary tuberculosis following a period of medical management has emerged in the past ten years as the most useful of the surgical procedures. In most published series, good results are reported in 80 to 90 per cent of the cases, with a mortality rate of 5 per cent or less.

Well-supervised medical therapy, careful preoperative evaluation, and operative management have all served to lower the mortality even further in individual series. As surgical experience in the various groups and clinics has enlarged, the complications related to operative technique have become less. However, postoperative problems related to the residual pleural space, persistent air-leak, and bronchial stump fistula remain elusive in their solution. Many factors enter into the outcome of these complications, including drug-resistant tubercle bacilli, the extent of parenchymal disease, associated pulmonary and bronchial disease, and patient's degree of over-all resistance to the disease.

In an attempt to overcome these uncontrollable factors in certain candidates for resectional surgery, the writers elected to perform an upper-stage thoracoplasty two to three weeks prior to the resection in 30 selected cases. This was done on the premise that, should a pleural space or bronchial stump complication follow post resection, the resultant morbidity would be more easily corrected.

The present report is not a discussion of the merits of thoracoplasty in these 30 cases as a method of avoiding overdistention of remaining lung tissue and its relationship to reactivation of tuberculous foci, but rather a discussion of the immediate post-resection problems of: residual pleural space, empyema in this space, bronchopleural fistula, and atelectasis of the remaining lung tissue. Evidence that a problem exists may be seen from the fact that 76 of 356 resections performed on this service required one or more stages of post-resection thoracoplasty to reduce the volume of the residual pleural space or to combat dire complications such as bronchopleural fistula, either with or without empyema. In these cases of bronchopleural fistula the problems of additional major operations, in the presence of infection, drainage tubes, and an open bronchial stump, are sometimes most taxing both to the surgeon and the patient.

It may be seen, therefore, that there are three possible approaches to the problem: (*1*) pre-resection thoracoplasty in selected cases, (*2*) concomitant thoracoplasty in selected patients who can tolerate the procedure, (*3*) post-resection thoracoplasty after the complication develops or seems likely to develop.

Review of Literature

Day and associates (1) report the development of bronchopleural fistula and empyema in 13 per cent of 202 resections; it was the major cause of death. They emphasize the importance of the bronchial stump difficulties in both the early and the late complications from resection. Wareham (2) recently stated that in 41 of 136 partial resections for tuberculosis there was a persistent pleural space twenty-one or more days after operation. In 14 of the cases a bronchopleural fistula developed and was associated with the presence of drug-resistant tubercle bacilli in 10 instances. Only 2 of these 10 patients are clinically well; 4 improved after further surgery; and 4 are dead. Twenty-seven patients with asymptomatic pleural spaces made good recovery either with or without further surgery. Wareham and his associates conclude that a conservative nonoperative approach for residual pleural space may be used if the patient is carefully observed, constitutional or respiratory symptoms are absent, and there is progressive shrinkage of the residual space on serial roentgenograms. The sputum must be negative for tubercle bacilli, and effective drug therapy available. If these criteria cannot

[1] From the East Tennessee Tuberculosis Hospital, Old Tazewell Pike, Knoxville, Tennessee.

[2] Presented before the Medical Session, as part of *Section 6*, at the annual meeting of the American Trudeau Society, Kansas City, Missouri, May 8, 1957.

be met, then early operative intervention for space obliteration is recommended.

Cooley and associates (3) report on 189 resections in which bronchopleural fistula developed in 8 cases; empyema developed in 4; and a persistent air space in 13 cases. Twenty-five surgical procedures were utilized: 13 thoracoplasties, 5 decortications, 4 thoracotomies with stump closure plus thoracoplasty, and one bronchial stump excision.

Jones and Robinson (4) report no bronchopleural fistula in a series of 88 cases "protected" with streptomycin, but observed 11 fistulas in 123 resections with no streptomycin. Thus, resection in the patient with drug-resistant organisms should constitute a much greater hazard in the development of postoperative complications.

The role of endobronchial tuberculosis in the development of post-resection bronchopleural fistula is well known. The incidence of endobronchial tuberculous lesions in resected specimens is markedly lowered by antimicrobial therapy, as shown in a recent article by Gordon (5).

Murphy and associates (6), in their excellent paper on the problem of post-resection bronchopleural fistula, report a mortality rate of 30 per cent in patients with this complication in the years 1945 to 1950 and a mortality rate of 16.6 per cent in a recent group of patients more recently operated upon. They report a marked increase in morbidity in patients surviving bronchopleural fistula. They emphasize efforts to avoid bronchopleural fistula by means of careful case selection and preoperative treatment.

Gale and associates (7) had 18 operative deaths in 325 cases of resection, 5 of which were due to bronchopleural fistula and empyema. It is noteworthy, however, that, of six deaths after resection for thoracoplasty failure, none were due to this complication. They have used thoracoplasty concomitantly in 47 cases. However, they state that in 65 per cent of their resections the general condition of the patient would not permit the concomitant thoracoplasty, so it was deferred for six to twelve days. Likewise, Conklin and co-workers (8) did concomitant resection and thoracoplasty in 38 carefully selected cases, with one fatality. No patients developed bronchopleural fistula. The discussion which followed the paper by Conklin and associates brought out two pertinent points: (*1*) that the great paradoxical motion of the chest wall increases the morbidity, and (*2*) that frequently after an extrapleural upper lobe resection inadequate tissue remains to close the pleural space.

Observations

Pre-Resection Thoracoplasty (30 Patients)

After having observed the relatively benign postoperative course of patients who have had resections because of "thoracoplasty failure," it seemed reasonable to assume that, if an elective stage of pre-resection thoracoplasty could be performed in selected cases, the postoperative pleural and bronchial stump problems might not be as troublesome. Thus, a first-stage thoracoplasty was done, removing ribs 2 and 3 entirely and the posterior two-thirds of rib 4 with apicolysis under rib 1. At the stage of resection three weeks later, the posterior two-thirds of rib 5 was removed and the resection performed (figures 1, 2, 3).

Indications for pre-resection thoracoplasty: The indications for a pre-resection stage of thoracoplasty in 30 patients are shown in table 1. As may be seen in the table, this is a selected group of patients in whom post-resection complications would be highly probable. Many of the patients had more than one of the accepted indications for this procedure. The patients ranged in age from twenty-three to fifty years, with an average age of forty years. There were 16 males and 14 females.

Extent of resection: There were 18 resections on the right side and 12 on the left, 29 patients having at least a lobectomy or more. The other patient in this group had a tuberculous empyema and at resection required a wedge resection and decortication. The extent of resection in the 30 patients with pre-resection thoracoplasties were: lobectomy, 7; lobectomy and segmentectomy, 15; pneumonectomy, 4; bilobectomy, 2; bilobectomy and segmentectomy, 1; wedge and decortication (tuberculous empyema), 1.

Complications: Post-resection complications were many, as would be expected in this highly selected group in which trouble was anticipated. However, it is noteworthy that 12 cases were entirely uncomplicated. The complications in this group were as follows: retained secretions, 8; bronchopleural fistula with empyema, 6; chest wall bleeding, 2; elective tracheostomy, 4. Some of the patients had all of these complications.

Results

There were three deaths in this group, only one of which was related to bronchopleural fistula;

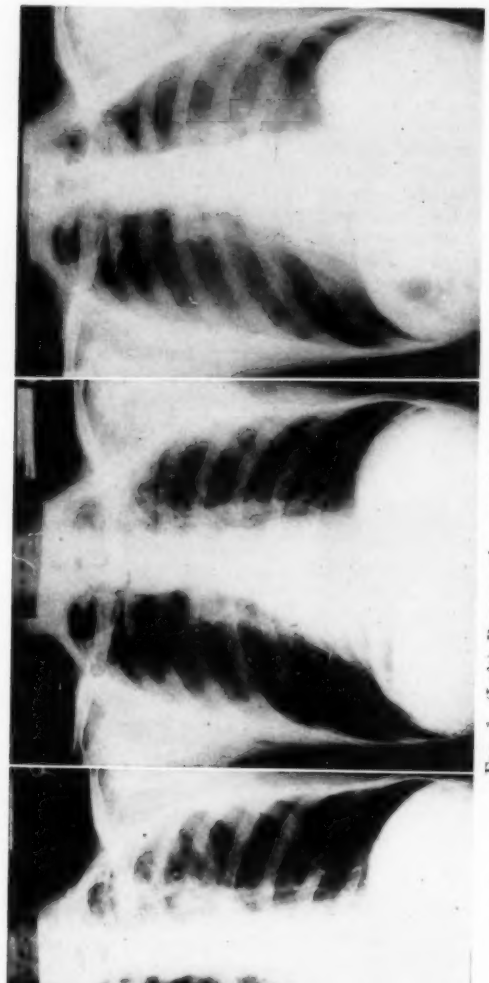

FIG. 1. (Left) Preoperative roentgenogram.
FIG. 2. (Center) Roentgenogram obtained after pre-resection thoracoplasty.
FIG. 3. (Right) Roentgenogram obtained after resection and thoracoplasty.

TABLE 1
INDICATIONS FOR PRE-RESECTION THORACOPLASTY
30 Patients

Large cavity	13
Endobronchial tuberculosis	11
Associated pulmonary emphysema	9
Extensive parenchymal tuberculosis	9
Drug resistance	8
Tuberculous bronchiectasis	6
Destroyed lung	4
Tuberculous empyema	1
Diabetes and tuberculosis	1

this death resulted from a massive hemorrhage six weeks post resection. One of the other two fatalities was related to pulmonary insufficiency on the second post-resection day and the other was due to a massive air embolism during a transfusion accident on the third post-resection day. The results were considered good in 24, with poor results in 3. Two of these have sputum positive for tubercle bacilli, are still actively under treatment, and will require further surgery. All 3 with poor results had bronchopleural fistulas and empyema.

Post-Resection Thoracoplasty (76 Patients)

In 76 of 356 resections it was necessary to do a post-resection thoracoplasty for one or more of the indications shown in table 2. These operations were performed in order to obliterate a post-resection pleural space or to control bronchial stump complications. It may be noted in table 2 that bronchopleural fistula with empyema was the indication in 17 patients (22 per cent), whereas bronchopleural fistula without empyema was present in 5 patients. Empyema was present in 3 patients. In some patients there was more than one indication for thoracoplasty.

Extent of resection: The extent of the resection in 76 cases requiring a post-resection thoracoplasty was as follows: lobectomy, 22; lobectomy and segmentectomy, 14; pneumonectomy, 24; bilobectomy, 2; segmentectomy, 3; bisegmentectomy, 7; trisegmentectomy, 3; wedge resection, 1. It may be seen that in only 11 of the 76 cases was less than a lobe resected. Forty-one of the resections were on the left and 35 on the right side.

Complications: The post-resection complications in this group were numerous, as might be expected; all of the patients required further surgical therapy to correct the complications. The complications were as follows: retained secretions, 10; bronchopleural fistula with empyema, 17; empyema, 3; residual pleural space, 24; excessive bleeding, 7; wound infection, 4; cor pulmonale, 2; pulmonary embolism, 1; spread of the tuberculosis, 1; massive subcutaneous emphysema, 1. Thus, the principal complications were persistent residual pleural space and bronchopleural fistula with empyema and retained secretions. These accounted for 51 of the 76 cases in which complications developed.

Results: Post-resection thoracoplasty: The late results in this group of 76 patients requiring post-resection thoracoplasties reveal 73.7 per cent with inactive disease and 26.3 per cent with active tuberculous disease. One surgical death related to bronchopleural fistula and empyema occurred six months post resection. Two others in this group had later deaths not related to surgery. Six patients remain with infectious sputum, 4 of whom are in the hospital and will need further surgery; all have had bronchopleural fistulas and empyema. Thus, of 17 patients with bronchopleural fistulas and empyema, the results in 10 are good; 6 of the patients have active disease; one is dead.

Resections Only (280 Resections—275 Patients)

In 280 resections in 275 patients with tuberculosis there was no need for thoracoplasty in order to control space difficulties or complications. However, pneumoperitoneum postoperatively was used in 9 instances and open thoracotomy was used in 8 cases to control air leaks and to perform a decortication.

Extent of resection: The extent of resection in the 280 cases not requiring thoracoplasty is as follows: lobectomy, 114 (11 patients having had associated wedge resection); lobectomy and segmentectomy, 14; bilobectomy (1 with associ-

TABLE 2
INDICATIONS FOR POST-RESECTION THORACOPLASTY
76 Patients

Residual pleural space	32
Pneumonectomy space	23
Bronchopleural fistula and empyema	17
Bronchopleural fistula	5
Empyema	3
Residual disease after resection	3

ated segmentectomy), 7; pneumonectomy, 7; segmentectomy, 77; bisegmentectomy, 20; trisegmentectomy, 4; wedge resection, 25.

Complications: The complications in 280 resections not requiring thoracoplasty are as follows: retained secretions, 8; bronchopleural fistula with empyema, 1; bronchopleural fistula, 6; prolonged air-leak, 40; empyema, 2; subcutaneous emphysema, 2; cor pulmonale, 2; thrombophlebitis, 3; pulmonary embolism, 1; pulmonary edema, 3; hemorrhage, 9; spread of the tuberculosis, 10; cerebral embolus, 1; pericarditis, 1.

Results: Of the 275 patients not requiring thoracoplasty, the tuberculosis in 210 is inactive and in 58 it is active. There were 3 surgical deaths and 4 later deaths. Sputum was positive for tubercle bacilli in 39 patients at the time of operation and was positive in only 4 after the resection.

Comparison of Results in the Three Groups

In a series of 386 resections, a modified thoracoplasty was performed in 106 instances to control real or anticipated pleural space problems. Thus, in 27.4 per cent of all resections performed, multiple stages of surgery were required.

The obvious reasons for space difficulties are: insufficient lung volume remaining to fill the residual pleural space; prolonged air leak caused by a small bronchopleural fistula without empyema; bronchopleural fistula with empyema; and empyema. The first of these is rather obvious and the quantitative dividing line used in the present series was whether a lobectomy or more was performed.

It will be noted in figure 4 that, in the pre-resection thoracoplasty group, in only one case was less than a lobectomy performed; also that, of 106 patients requiring thoracoplasty (either before or after resection), a lobe or more was resected in 95 patients. The second cause is related to a residual pleural space with persistent air leak with the potential danger of empyema as long as it is present. The present writers have followed the well-established principle of obliterating a residual space when it is present and represents a potential danger. The third reason related to bronchopleural fistula is the most serious one and adds materially to the mortality and morbidity when it does occur.

It may be seen in table 3 that, in 106 cases of resection in which thoracoplasty was performed as an added operation, bronchopleural fistula with empyema was the complication in 23 patients, or 21.7 per cent of the group. Six patients in whom this complication had been anticipated had a stage of pre-resection thoracoplasty; the remaining 17 patients developed the complication and then had corrective surgery. Finally, empyema alone was a rather infrequent complication and generally occurred after prolonged tube drainage in an attempt to obliterate a

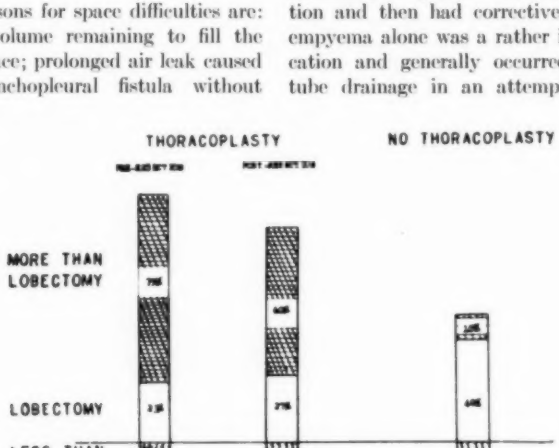

FIG. 4. Graphic presentation of figures 1, 2, and 3.

TABLE 3

COMPLICATIONS AFTER RESECTION

	Pre-Resection Thoracoplasty	Post-Resection Thoracoplasty	Resection—No Thoracoplasty
Retained secretions	8 (27%)	10 (13%)	8 (2.8%)
Bronchopleural fistula and empyema	6 (20%)	17 (22%)	1 (0.3%)
Bronchopleural fistula	—	—	6 (2.1%)
Empyema	—	3 (3.9%)	2 (0.7%)
Prolonged air-leak	—	—	40 (13%)
Residual pleural space	—	24 (30.0%)	—
Intrapleural bleeding	2 (6.6%)	7 (9.0%)	9 (3.2%)
Spread	—	—	10 (3.5%)
Wound infection	—	4 (5.2%)	—
Miscellaneous	—	5 (6.5%)	13 (4.6%)

residual pleural space, or followed postoperative intrapleural hemorrhage with a residual clotted hemothorax.

A comparison of the types and incidence of the complications in the three groups is shown in table 4. From these data in 386 cases of resection, it is believed that a pre-resection stage of thoracoplasty is helpful in certain selected cases in the post-resection phase. In 76 cases grouped as post-resection thoracoplasty, it is believed that the 17 cases complicated with bronchopleural fistula and empyema could have been better handled by one or more stages of pre-resection thoracoplasty.

The apparent high percentage of patients having active disease in the resection–no thoracoplasty group is explained by the fact that a number of the patients were still in the hospital or still under active treatment, having been operated upon less than a year prior to the study.

The three surgical deaths in the pre-resection thoracoplasty group were related to: hemorrhage from major vessel erosion six weeks after resection, complicated by bronchopleural fistula and empyema; massive air embolism during transfusion accident postoperatively; and pulmonary insufficiency two days postoperatively. The one surgical death in the post-resection—thoracoplasty group was due to pulmonary embolism on the fifth postoperative day. The three surgical deaths in the resection–no thoracoplasty group resulted from pulmonary insufficiency two weeks postoperatively, an anesthesia death on the operating table, and pulmonary insufficiency on the eighth postoperative day.

The criteria used for the selection of patients to perform an elective stage of pre-resection thoracoplasty are shown in table 5. A large number of the patients had all of the accepted criteria and represented cases that, from previous experience, would have been expected to develop complications in the post-resection phase.

TABLE 4

RESULTS (386 RESECTIONS)

	Pre-Resection Thoracoplasty	Post-Resection Thoracoplasty	Resection—No Thoracoplasty
Inactive	24 (80%)	55 (72%)	210 (75%)
Active	3 (10%)	18 (25%)	58 (20%)
Deaths—surgical	3 (10%)	1 (1%)	3 (1%)
Deaths—later	—	2 (2%)	4 (1%)

TABLE 5

CRITERIA FOR PRE-RESECTION THORACOPLASTY

1. Resection of more than upper lobe
2. Infectious sputum at time of surgery
3. Drug resistance—clinically or bacteriologically
4. Maximal breathing capacity of less than 50 per cent of predicted value
5. Associated obstructive pulmonary emphysema
6. Endobronchial tuberculosis at lobar bronchus level
7. Extensive "healed" disease to remain after resection

DISCUSSION

From the experience presented it would appear that a candidate for resectional therapy who, from the preoperative assessment, has *more than one of the criteria present* (table 5) should have a pre-section stage of thoracoplasty rather than a post-resection thoracoplasty. The problem is to decide prior to resection which patient will need a thoracoplasty and to perform this as an elective

pre-resection procedure rather than as a mandatory post-resection operation. It might have been anticipated that all of the 30 patients selected for the pre-resection thoracoplasty group by these criteria would have difficulty. In actuality, the course in 12 patients (40 per cent) of the group was entirely uncomplicated. It is believed that these 12 patients had their good results as a result of the pre-resection thoracoplasty.

From this experience it should be emphasized that a pre-resection thoracoplasty is no panacea and that complications do develop post resection; however, they are more easily controlled, and it is believed that the end result is better. It is the present practice to wait six weeks between the pre-resection thoracoplasty and the resection stage. This interval gives the patient a more stable chest wall in the post-resection phase and leads to less trouble with retained secretions from paradoxical motion of the chest wall.

Summary

The conclusions from this study indicate that a stage of pre-resection thoracoplasty is valuable in a selected group of patients with advanced disease or disease refractory to medical management. It is in this group that bronchopleural fistulas and empyema are prone to develop, and a pre-resection thoracoplasty limits the empyema space should one occur. It is recommended that six weeks elapse between the pre-resection thoracoplasty and the resection.

The fact that, of the total number of 356 resections, 27.4 per cent of the patients required some type of thoracoplasty (due to actual or potential space complications) would indicate that selective anticipation of this need before resection would eliminate numerous post-resection difficulties.

Sumario

Las Indicaciones y el Empleo de la Toracoplastia de Pre y Post-Resección en la Tuberculosis

Indican las conclusiones de este estudio que una etapa de toracoplastia de pre-resección resulta valiosa en un grupo escogido de sujetos que tienen enfermedad avanzada o refractaria a la asistencia médica. Este grupo es el más propenso a manifestar fístulas pleurales y empiema y una toracoplastia pre-resección limita el espacio que ocupa el empiema, si es que se presenta. Recomiéndase que se dejen transcurrir seis meses entre la toracoplastia pre-resección y la resección.

El hecho que, del total de 356 resecciones, 27.4 por ciento de los enfermos requirieron alguna forma de toracoplastia (debido a complicaciones existentes o potenciales en cuanto a espacio) indicaría que la anticipación selectiva de esta necesidad antes de la resección eliminaría numerosas dificultades después de la operación.

Resume

Les indications et l'emploi de la thoracoplastie pré-résectionnelle et post-résectionnelle dans la tuberculose

Les conclusions fournies par cette étude indiquent que dans un groupe sélectionné de sujets présentant une tuberculose avancée ou réfractaire au traitement médical, l'étape de la thoracoplastie est favorable. C'est dans ce groupe que les fistules bronchopleurales et l'empyème sont aptes à se développer. Un délai de six semaines entre la thoracoplastie pré-résectionnelle et la résection est préconisé.

Le fait que, sur un nombre total de 356 résections, 27, 4% des patients étaient justiciables d'un type quelconque de thoracoplastie (en raison de complications cavitaires existantes ou potentielles) indique qu'il y a lieu de prévoir la nécessité de cette intervention de choix avant la résection, ce qui éliminerait de nombreux problèmes post-résectionnels.

Acknowledgment

The writers wish to thank Mrs. Frances Banner and Miss Barbara Snyder for their assistance in the preparation of the manuscript.

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IMMUNOLOGIC ASPECTS OF PULMONARY DISEASES[1, 2]

A Review

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In 1904 Ehrlich (1) formulated his theory of molecular receptors complementary to haptophoric groups to explain antibody-antigen reactions, and shortly thereafter Von Pirquet (2) recognized the potentially adverse effect of these interactions. During the last ten years numerous contributions to our understanding of immunologic processes have been made. It seems timely to review these as they relate to pulmonary disease in general and to bronchial asthma and hypersensitivity granuloma of the lung in particular.

The formation and life span of antibodies have been the subject of extensive investigation. It is now well established that antibodies are not "adapted" globulins, but are formed *de novo* (3, 4) by cells, and the preponderance of opinion today is that plasma cells and/or lymphocytes are their manufacturers (5, 6). Thus, all antibodies are originally located intracellularly. Upon release into the circulation, the antibody combines with antigen to form an antibody-antigen complex, thus neutralizing the antigen (7). During this phase, its demonstration *in vitro* is difficult. If there are more antibodies available than needed for combination with the antigen, the free or excess antibodies will readily combine *in vitro* with homologous antigens and be easily demonstrable (7, 8).

The life span of circulating antibodies varies widely with animal species, antigen, and method of immunization. In children, their half life has been reported by Dixon (9) as 20.3 ± 4.0 days; in adults, 13.1 ± 2.8 days. Circulating antituberculous antibodies showed a continued high titer seven to nine weeks after rabbits were inoculated with live or heat-killed tubercle bacilli (10). That antibodies responsible for tuberculin skin hypersensitivity in untreated persons persist for many years may be deduced from the data reviewed by Rich (11). Chemotherapy apparently shortens the life span of these antibodies, as indicated by reversal of the Mantoux test in 35 per cent of 60 tuberculous children within an average period of sixteen months (12).

To explain the long-continued manufacture of antibodies, two hypotheses have been advanced: (*1*) that the antigen persists in the host cell (13), and (*2*) that a permanent change has taken place in the molecular architecture of the antibody-producing cell. This results in a change of the "template" which is responsible for the continued output of globulins carrying a combining site for a specific antigen (14).

The continued presence of antigenic bacterial and fungal polysaccharides has been amply demonstrated in experimental animals (15–17). In man, two significant examples may be cited. Dextran, given as a blood expander, is stored in some patients in reticuloendothelial cells and antibodies are formed against it (18). A much more serious effect of such continued intracellular harboring of antigen has been brought into focus by Felton and co-workers (19). They demonstrated that a single large dose of pneumococcal polysaccharide will produce immunologic paralysis in mice, that is, inability to produce antibodies. This may last for at least fifteen months. Based on a study of tissue from 125 autopsy cases, these writers suggest that "accumulations of polysaccharide antigen in human tissues may influence susceptibility to pneumococcus infection and, hence, perhaps be a contributing factor in the incidence of pneumonia" (20).

The primary function of the antibody is defense, but in some persons additional phenomena develop. This is illustrated by the study of Kuhns and Pappenheimer (21*a* and *b*), who demonstrated that 30 per cent of 131 subjects hyperimmunized with diphtheria toxoid developed skin hypersensitivity of the immediate type in addition to protective precipitating antibodies. These two activities reside in two different gamma-

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[2] Presented at the annual meeting of the California Trudeau Society, San Mateo, California, April 11, 1958.

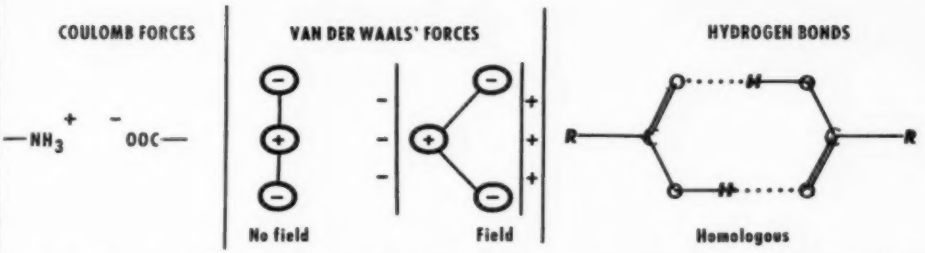


FIG. 1. Forces combining antibody with antigen. The hydrogen bonds as illustrated are a "double bond" between two carboxyl groups, each of which donates one proton (hydrogen). Such groups are present in proteins; for instance, gamma-globulin and albumin (antigen).

globulin fractions. The fact that some recipients develop one, while others can develop two different antibodies to the same type of immunization, represents a transition to those instances in which the harmful effects of antibody-antigen interactions supervene and produce disease. These immunologic diseases have recently been comprehensively reviewed by Raffel (22). Therefore, only the mechanism underlying their pathophysiology, as far as it has been elucidated, will be discussed here.

It was recognized early that it would be essential to have an understanding of: the molecular properties of both antibody and antigen which facilitate their combination; the forces holding them together; and the molecular changes arising from their interaction. The extensive studies by Landsteiner (23) and Pressman and associates (24) have elucidated many molecular groupings responsible for antigenicity, but information on the molecular structure of the combining site of the antibody has been wanting. As the combining forces (25), electrostatic, Van der Waal's attraction, and hydrogen bonds (figure 1) have been suggested. In 1955, Singer and Campbell (26), on the basis of thermodynamic calculations, concluded that hydrogen bonds are formed between carboxyl groups of both the antibody and the antigen. This is an important clue to the combining site of the antibody. Data indicating that such binding also occurs between antituberculous gamma-globulin and tuberculoprotein have been obtained at this laboratory (27). Both electrostatic forces and hydrogen bonds are considerably stronger than Van der Waal's attraction. A revealing example of this difference has been contributed by Pressman (28). He employed haptens of similar shape and size which were complementary to identical antibodies but differed in the presence or absence of an electric charge. Experimentally, he observed a factor of 8 between the relative combining constants of these two haptens with the antibody.

The effect of a firm combination between antibody and antigen is the formation of a large complex resistive to degradation. As early as 1934, Letterer (29) suggested that amyloid represents the deposition of an antibody-antigen complex. Vazquez and Dixon (30) employed the fluorescent-labeled antibody technique to demonstrate that amyloid contains gamma-globulins. This is suggestive evidence that amyloid disease represents the deposition of an antibody-antigen complex.

As to the significance of antibodies in tuberculosis, opinions differ. The concept has been held for many years that, with the exception of their role in skin hypersensitivity, antibodies are of no consequence in tuberculous disease. This opinion is largely based upon the failure to detect circulating antibodies in nearly 40 per cent of tuberculous patients (31), and the lack of beneficial effect from the transfusion of immune serum upon the experimental disease in animals (32). Reinvestigation of the problem of the apparent absence of circulating antibodies in tuberculous patients by the present writers and co-workers (33) revealed that antibodies are present in nonreacting tuberculous sera and can be demonstrated after fractionation of the serum proteins. Recently, Parlett and associates (34) also were able to show the presence of antibodies in 48 of 52 tuberculous sera.

In considering the function of these antibodies Lurie's demonstration of the role of the monocyte in resistance to tuberculosis must first be men-

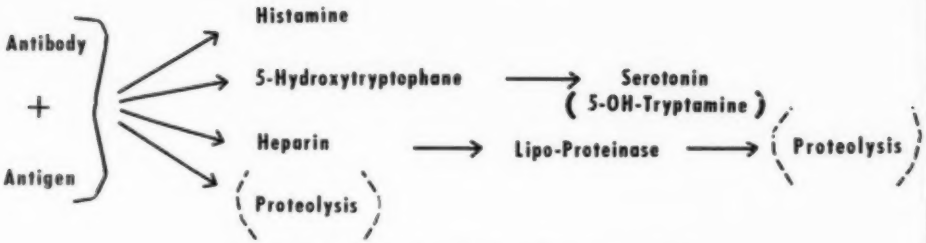


FIG. 2. Side effects of antibody-antigen combination.

tioned (35). The mechanism enabling the monocyte of an immune or resistant rabbit to inhibit the multiplication of the phagocytosed tubercle bacilli is still unknown (35, 36). The studies by Fong and co-workers (37) suggest that monocytes maintained in immune serum are better able to resist the degenerative effect of harbored tubercle bacilli. A recent report by Zitrin and Wasz-Höckert (38) supports the view that immune globulins assist the experimental animal in its struggle against tuberculous infection. However, there are contradictory reports on this problem (39, 40), and further clarification is needed.

The undeniably beneficial effect of immunization in tuberculosis suggests that some mechanism is created or becomes better adapted to resist the deleterious effect of tubercle bacilli. Since in many instances antibodies are synonymous with anti-enzymes (41), the immunizing effect of living bacilli or bacillary fragments exhibiting enzymatic activity (42) may be explained on the basis of the host's cells acquiring means, i.e., anti-enzymes, to resist more effectively the aggressive enzymes of the parasite.

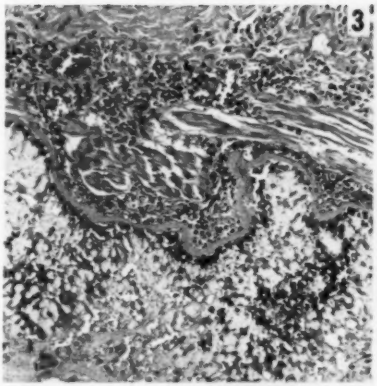
The harmful sequelae of antibody-antigen interaction previously mentioned can be demonstrated and correlated to the pathogenesis of the lesion in two pulmonary diseases, namely, extrinsic bronchial asthma and hypersensitivity granuloma.

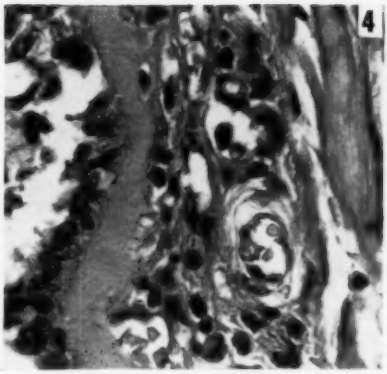
A series of reactions consequent upon antibody-antigen combination underlie an asthmatic attack. Their elucidation, initiated by Dale and Laidlaw's suggestion of the release of a histamine-like substance (43), has furnished the data presented in figure 2: The interaction of antibody and antigen in the presence of platelets (44) or basophilic leukocytes (45) results in the release of histamine, serotonin, and heparin. There is an accompanying intense proteolysis, but the problem of which of these processes is the causal event is as yet unsolved (45–47).

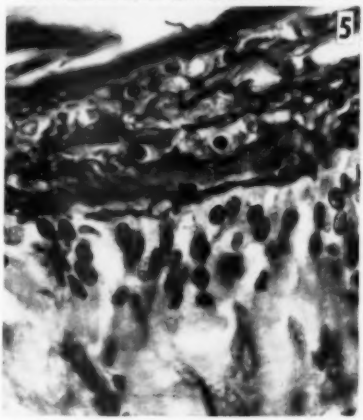
The pulmonary histopathology of a 23-year-old white male who died during an asthmatic attack is illustrated in figure 3. The distended bronchus is filled with mucus and numerous eosinophils; a thickened hyalinized basilar membrane is present; prominent muscle bundles and innumerable eosinophils are seen throughout all layers of the bronchial wall.

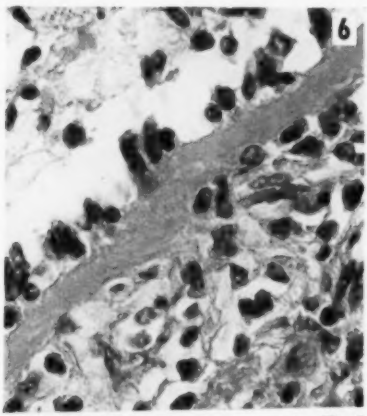
The excessive mucous production with the concomitant impairment of the ciliary activity has not been identified with any part of the antibody-antigen reaction. It is known, however, that the mucus in asthma is highly viscous, much more so than the secretions observed in infectious bronchitis. This may be the result of an increased concentration of mucoproteins. The usual amount of that protein is 1 to 2 per cent, but a seemingly small increase to 3 or 3.5 per cent rapidly increases its consistency (48). It may be surmised that heparin, because of its affinity for certain polysaccharides such as dextran sulfate, combines with the mucoproteins and thereby gives rise to a secretion of different character.

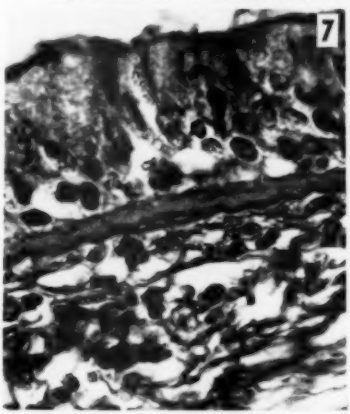
The origin of the numerous eosinophils and their function has been restudied by Godlowski (49). He has shown that many of the eosinophils are formed locally and represent an adaptation phenomenon. Apparently, catabolism of the large antibody-antigen complex requires additional enzymes, and the eosinophilic granules are their morphologic counterpart. The degradation of proteins may follow an unusual pathway (49), the resulting abnormal split products causing tissue damage. In addition, eosinophils contain a steroid-like antihistaminic substance (50, 51) and this makes their presence doubly purposeful. The experimental work of Herxheimer (52) and of Huković and associates (53) suggests that











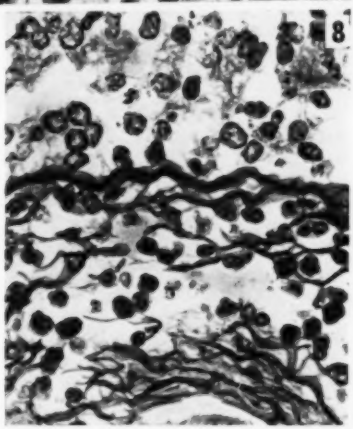


FIG. 3. Bronchus of 23-year-old male who died during asthmatic attack. (Hematoxylin and eosin, ×75)
FIG. 4. Hyalinized basilar membrane of bronchus illustrated in figure 3. (Hematoxylin and eosin, ×560)
FIG. 5. Same as figure 4. (Wilder's reticulum stain, ×560)
FIG. 6. Part of bronchial wall of 43-year-old female who died in status asthmaticus. Hyalinization of basilar membrane. (Hematoxylin and eosin, ×560)
FIG. 7. Same as figure 6. (Wilder's reticulum stain, ×560)
FIG. 8. Another part of bronchial wall of patient illustrated in figures 6 and 7. (Wilder's reticulum stain, ×560)

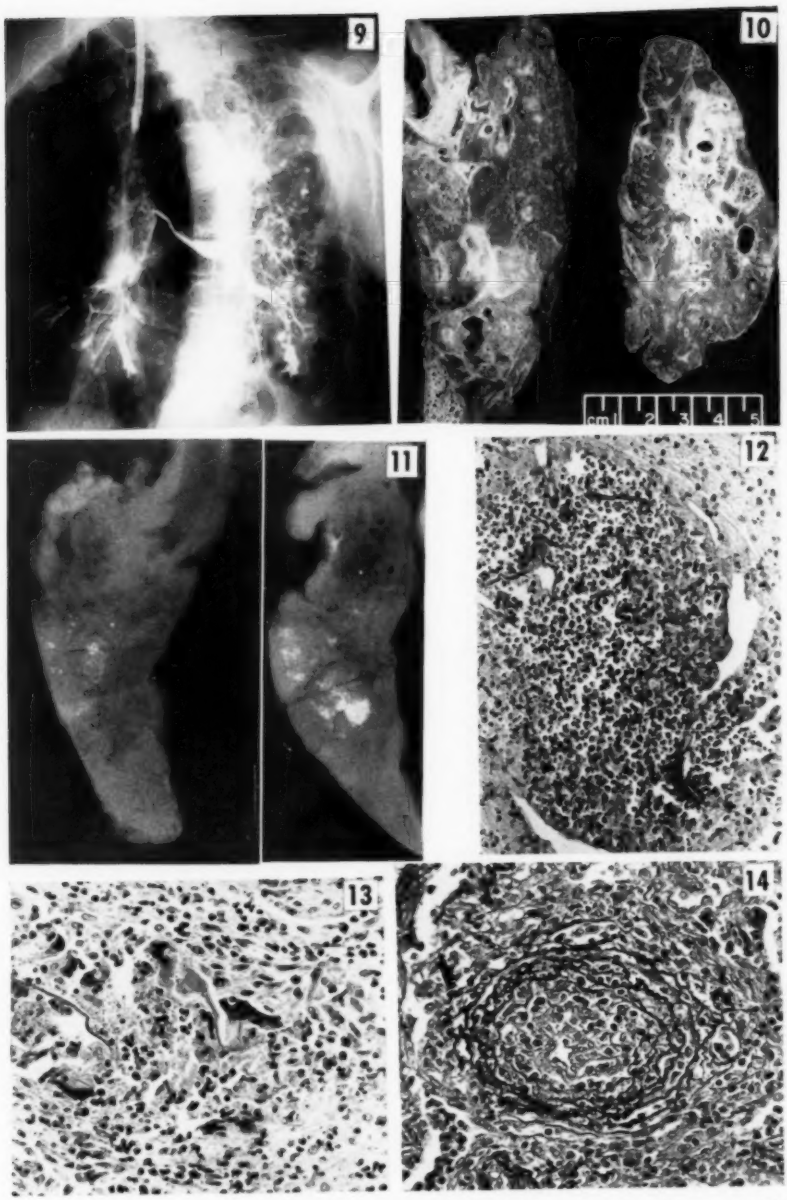


FIG. 9. Bronchogram of 45-year-old male, demonstrating bronchiectasis. Note absence of other radiopacities.

FIG. 10. Sectioned surface of left lower lobe obtained by lobectomy.

FIG. 11. Roentgenograms of slices of lung illustrated in figure 10 demonstrating radiopaque material.

FIG. 12. Histologic section of lung (*see* figure 10). Masses of eosinophils surrounding fibers undergoing fibrinoid degeneration. (Hematoxylin and eosin, ×216)

FIG. 13. Same case as figures 9 to 12. Degenerated fibers partly engulfed by multinucleated giant cells. (Hematoxylin and eosin, ×300)

FIG. 14. Same case as figures 9 to 13. Vessel with fragmented elastic lamina occluded by granulomatous tissue. (Elastica van Gieson's stain, ×300)

5-hydroxytryptamine or serotonin induce smooth muscle contraction. As serotonin is released as a result of antibody-antigen reaction, this may account for the marked contraction of the smooth bronchial muscles which is so prominent a feature of bronchial asthma.

The pathogenesis of the hyalinization of the basilar membrane is unknown. The appearance of a hyalinized basilar membrane when stained with hematoxylin and eosin is illustrated in figure 4. A corresponding silver stain (figure 5) demonstrates that the reticulum fibers of the basal membrane are preserved, although thickened, and that a substance has been laid down between them. Similar features are seen in sections of the bronchus of a 43-year-old female who died in status asthmaticus (figures 6 and 7). In another part of the same bronchus, only the thickening of reticulum fibers is present (figure 8). It may be that this hyalinization is the result of a deposition of proteins, as suggested by Müller's experiments (54) and as demonstrated in the formation of amyloid (30). Such hyalinization is not present in the early stage of the disease, for it was not formed in the lung of a child who died during the first attack (55).

The effect of hyalinization of the basilar membrane upon the elasticity or compliance of the lung is important physiologically. According to Scherrer and co-workers (56), the viscosity of the lung in asthmatics is five times the normal. If the hyalinized membrane is visualized as a continuous pipelike structure, and not as the single ring of the microscopic preparation, then it becomes obvious that the bronchial wall must be considerably stiffened.

Biopsies taken during the asthmatic attack reveal edema of the bronchial mucosa and submucosa (57). It is not known at present whether histamine or some other protein degradation product (49) is responsible for this edema, but undoubtedly it is a sequela of the immunologic reaction.

Case Report

A case of hypersensitivity granuloma of the lung serves to demonstrate further the tissue changes subsequent to antibody-antigen interaction. A 45-year-old male patient had a bronchography and subsequently a resection of the left lingula for bronchiectasis in 1951. In the spring of 1957, he re-entered the hospital because of the recurrence of symptoms. A bronchogram using propyliodine (Dionosil oily®) revealed bronchiectasis of the left lower lobe (figure 9). Surgery was performed thirteen days later and, in addition to bronchiectasis, tumor-like lesions were found (figure 10). Histologic studies demonstrated an extensive lipoid pneumonia as well as the usual bronchiectatic changes. The lipid material in the lesions was radiopaque (figure 11). In addition, collagen fibers undergoing fibrinoid degeneration and fragmented elastic fibers were seen (figure 12). These were partly engulfed by giant cells and surrounded by masses of eosinophils (figure 13). While many of the vessels in these areas had fragmentation and destruction of their elastic lamina with partial or complete granulomatous occlusion (figure 14), others showed only an intimal reaction such as that described by Ehrlich and Romanoff (58).

The presence of a necrotizing eosinophilic process associated with vascular lesions differentiates this necrotizing lesion morphologically from that of a toxic pneumonia, which occasionally follows the instillation of iodipin (59) or of water-soluble contrast material (60). The periarteritis nodosa-like changes characteristic of granulomas, reported by Churg and Strauss (61) in cases of severe bronchial asthma, and the central areas of acidophilic granular necrotic tissue surrounded by epithelioid cells seen in Löffler's syndrome (62) are absent in the lesions described above. This case, however, fulfills the criteria of allergic granulomas, as stipulated by Bohrod (63), namely, predominantly eosinophilic cellular infiltrate, fibrinoid swelling of fibers, and vascular changes. Whether the oil or the iodine compound was the sensitizing agent is not known, but the case offers an illustration of the powerful side effects to an allergic reaction.

Summary

Recent developments in the field of immunology have been reviewed with particular reference to pulmonary diseases. The persistence of relatively large amounts of antigenic material may produce paralysis of the immunologic defense.

As has been demonstrated recently, the physicochemical forces uniting antibody and antigen also involve hydrogen bonds. If the antibody-antigen combination is a very firm one, the resulting complex may be deposited in the tissues of various organs giving rise to changes; for instance, amyloid disease. Hyalinization of the basilar membrane of the bronchi in bronchial asthma is suggested to be due to a similar mecha-

nism. Eosinophilia in bronchial asthma is interpreted as an adaptation phenomenon and mucosal edema due to the formation of abnormal protein split products.

A case of hypersensitivity granuloma of the lung is reported, and its histology discussed in terms of immunologic reactions.

Sumario

Aspectos Inmunológicos de las Afecciones Pulmonares

Repásanse los recientes desenvolvimientos en el campo de la inmunología con referencia particular a las neumopatías. La persistencia de cantidades relativamente grandes de materias antigénicas puede producir parálisis de la defensa inmunológica.

Según se ha demostrado recientemente, las fuerzas fisicoquímicas que unen anticuerpo y antígeno también comprenden lazos de hidrógeno. Si la combinación de anticuerpo-antígeno es muy firme, puedo depositarse el complejo resultante en los tejidos, dando origen a alteraciones; por ejemplo, amiloidosis. Señálase que puede deberse a un mecanismo similar la hialinización de la membrana basilar de los bronquios en el asma bronquial. Se interpreta la eosinofilia del asma bronquial como fenómeno de adaptación y se atribuye el edema de la mucosa a la formación de productos anormales de disociación de proteínas.

Preséntase un caso de granuloma del pulmón por hipersensibilidad y se discute su histología en términos de reacciones inmunológicas.

Resume

Aspects immunologiques des maladies pulmonaires

Les récentes découvertes en immunologie sont passées en revue, sous l'aspect particulier des maladies pulmonaires. La persistance de substances antigènes en quantité relativement élevée peut produire la paralysie des défenses immunologiques.

Ainsi qu'on l'a récemment démontré, les forces physicochimiques unissant l'anticorps à l'antigène comportent également la liaison hydrogène. Si la combinaison anticorps-antigène est très solide, le composé formé peut se déposer dans les tissus de divers organes en entraînant certains changements, par exemple l'altération amyloïde. L'hyalinisation de la membrane basale des bronches dans l'asthme bronchique est attribuée à un mécanisme analogue. L'éosinophilie de l'asthme est interprétée comme étant un phénomène d'adaptation et l'oedème muqueux pourrait être dû à la formation de produits de dégradation protéïque anormale

Les auteurs rapportent une observation de granulome pulmonaire d'origine allergique, dont l'histologie est commentée en termes de réactions immunologiques.

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TISSUE CULTURE STUDIES ON RESISTANCE IN TUBERCULOSIS[1, 2, 3]

II. Monocytes from Normal and Immunized Guinea Pigs Infected with Virulent Human Tubercle Bacilli

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INTRODUCTION

In contrast to many infectious diseases in which the formation of specific antibodies has been shown to be one of the major factors in resistance, the development of antibodies in animals infected by tubercle bacilli has been demonstrated only in a few cases. The concept has been long advocated that acquired resistance in this disease for the most part depends upon cellular factors. However, Rich (1) was not able to show that leukocytes from immunized animals had any greater ability to inhibit the multiplication of, or resist the injury produced by, tubercle bacilli in tissue culture than did normal leukocytes. He used tissue culture procedures which did not permit observations beyond three or four days. Lurie (2) found that, when monocytes from immunized rabbits were infected by tubercle bacilli and transferred to the anterior chambers of the eyes of other rabbits, the rate of multiplication of those bacilli in the anterior chamber was slower than when leukocytes from normal rabbits were used.

In recent years, workers who have used improved tissue culture techniques have arrived at conflicting conclusions. Suter (3) believed that he was able to demonstrate a significant inhibition of the multiplication of tubercle bacilli by the monocytes from BCG-immunized guinea pigs in contrast to the multiplication in monocytes from normal animals. The addition of immune serum to such tissue cultures was said to produce no effect upon these observations. Mackaness (4), on the other hand, was not able to demonstrate any basic difference in the behavior of the tubercle bacilli in cells of immunized or control rabbits. Fong and associates (5) recently reported that a benefit from immune serum alone had been demonstrated. Suter and Mackaness (3, 4) counted the increases of intracellular bacilli as an end point while Fong and co-workers based their conclusions upon visible "toxic" changes in the cells; however, in this latter investigation the cells were studied for only three days.

Because of the fundamental importance of this question which has intrigued workers for years, the present writers have reinvestigated the problem. It was thought that the tissue culture method should be useful since it presents the opportunity to isolate cellular and humoral factors. It was also believed that important differences in the tissue culture techniques have led to the conflicting observations of recent workers.

EXPERIMENTAL METHODS

The methods are essentially those reported previously (6). Briefly, monocytes have been obtained from the peritoneal cavities of guinea pigs approximately ten days after the instillation of 1 ml. of mineral oil. This oil has been stated by the manufacturer to be essentially free of surface-active substances. The cells from immunized and normal animals are washed and measured, and equal quantities of cells are added to a series of test tubes. Comparable quantities of a homogenized and filtered suspension of tubercle bacilli are added to both normal and "immune" cells in amounts according to the measured amounts of cells present in that particular experiment.[4] In each experiment two levels of infection were established in both control and immune cells so that both light and heavy infections could be simultaneously studied in the control and immune cells. Samples of both groups of cells were also kept without infection.

Phagocytosis is allowed to take place in a streptomycin-free medium in a roller-tube apparatus

[1] From the Glockner-Penrose Hospital, Colorado Springs, Colorado.

[2] This investigation was supported by a research grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and research grant E 808 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

[3] With the technical assistance of Mrs. Jayne Gerbig and Mr. Gerald Lorentzson.

[4] The tubercle bacilli have been supplied by the courtesy of Drs. Gardner Middlebrook and M. L. Cohn of the National Jewish Hospital for Tuberculosis, Denver, Colorado.

at 37°C. for approximately one hour. Working under a hood, drops of the cell-bacilli suspensions are then placed on coverslips and mixed with guinea pig plasma and a small amount of thromboplastic substance. After a clot has formed, the coverslips are put into test tubes. At the same time, similar preparations are made on the side walls of roller tubes, both in clot and on glass. Nutrient feed consisting of 40 per cent normal guinea pig serum and 60 per cent Hanks solution, with 50 units of penicillin and 5 γ of streptomycin per ml. are added (7), and the tubes are incubated in a roller tube apparatus at 37°C. Representative coverslips are fixed in formalin at regular intervals or at times indicated by daily study of the roller tube side-wall preparations. The coverslips are stained by Kinyoun's technique. The percentage of monocyte infection and the number of bacilli per monocyte are counted on the stained and permanently mounted coverslips. The number of monocytes with intracellular cords or solid masses of bacteria and monocytes which are bursting with bacilli are also counted per low-power field. The number of monocytes which are visibly dead with and without bacilli is also estimated, but this has proved difficult.

Cells presumed to be mesothelial cells are readily separable from monocytes. Few at first, these cells rapidly multiply and form sheets of cells. The nuclear and cytoplasmic structures are quite different from those of monocytes. Even though these cells appear to contain bacilli in increasing numbers, no study of such cells has been made. All bacterial or cell counts and observations were confined to recognizable monocytes.

The tubercle bacilli used have been strains *G*, Petersen, and 5159 of H37Rv. Heat-killed bacilli have often been used as controls in experiments. The tubercle bacilli have been seven-day cultures in Dubos-Middlebrook medium. The bacilli have been emulsified in a Teflon homogenizer and have been filtered through a medium sintered glass filter. The resulting suspension in albumin-Tween® solution has been used without further washing or filtering in quantities of 0.05 to 0.2 ml. per 0.25 ml. of monocyte suspension according to the number of monocytes available in each particular experiment and the level of infection desired.

Guinea pigs have been immunized by intra- and subcutaneous infections of living BCG organisms or, more recently and more satisfactorily, by the air-borne infection of the animals with a catalase-negative 5159 strain of tubercle bacilli.[5] In all studies of "immune" monocytes, normal guinea pig monocytes served as side-by-side controls in each experiment.

[5] The air-borne infection chamber has been provided by the National Jewish Hospital for Tuberculosis, Denver, Colorado, through Drs. Gardner Middlebrook and M. L. Cohn.

Results

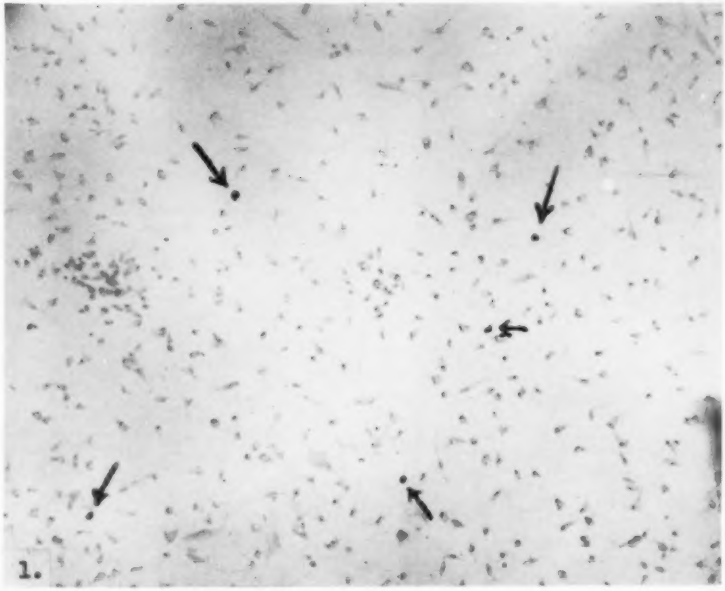
Phagocytosis of Virulent Bacilli by "Immune" and Normal Guinea Pig Monocytes

In repeated experiments it was not possible to detect any difference in the rate of phagocytosis of the living virulent tubercle bacilli between the cells of the immune and normal animals. With experience it was possible to arrange the concentration of bacilli and cells so that at the end of ninety minutes in the roller tube apparatus only rare extracellular tubercle bacilli remained. Sample smears prepared at ten, thirty, sixty, and ninety minutes failed to reveal any consistent increase in the rate of phagocytosis by the immune cells over that of the control cells, whether the immune cells were obtained from animals which had been previously desensitized or not or whether the cells were in immune or in control serum. More meticulous techniques for the study of phagocytosis may be necessary to reveal possible differences, and such a study is planned.

Lethal Effect of Virulent Tubercle Bacilli on "Immune" and Normal Guinea Pig Monocytes

With heavy infections, such as 60 per cent of all monocytes infected with perhaps 20 per cent of the cells containing more than 5 bacilli per cell, there is usually quite a high rate of monocyte death during the first twenty-four to forty-eight hours. With such an infection, perhaps 25 per cent of the cells will die within the first forty-eight hours. With light infections of 20 to 50 per cent of the cells infected, and with almost no cells which contain more than 5 bacilli, there is usually no greater initial cell death than in the uninfected controls (figure 1).

In no experiment was there any significant difference in the frequency or rate of initial cell death among infected normal cells in contrast to infected immune cells. Indeed, in heavy infections, more immune (and hypersensitive) monocytes appeared to die during the first forty-eight hours than did control cells. This difference was not noticed in lighter infections. The onset of cell migration which may indicate the rate of recovery from the trauma of the preparation of the cultures was as prompt in infected control as in infected immune cells. There was no greater granularity in the infected control cells than in the infected immune cells as seen on the living



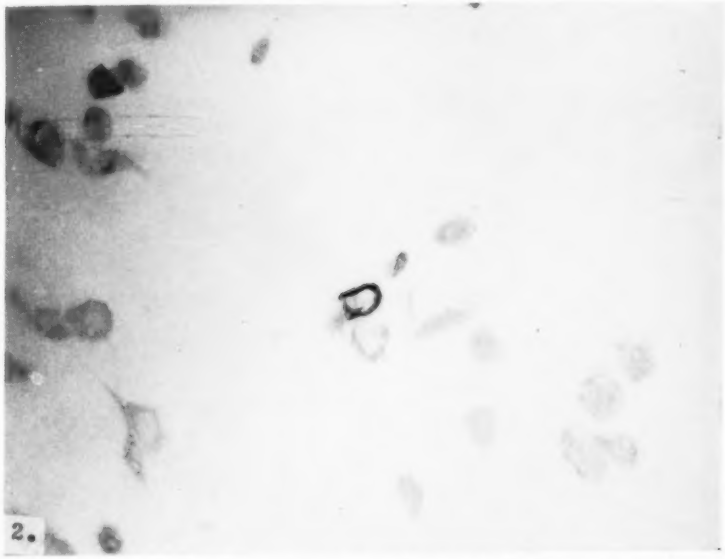
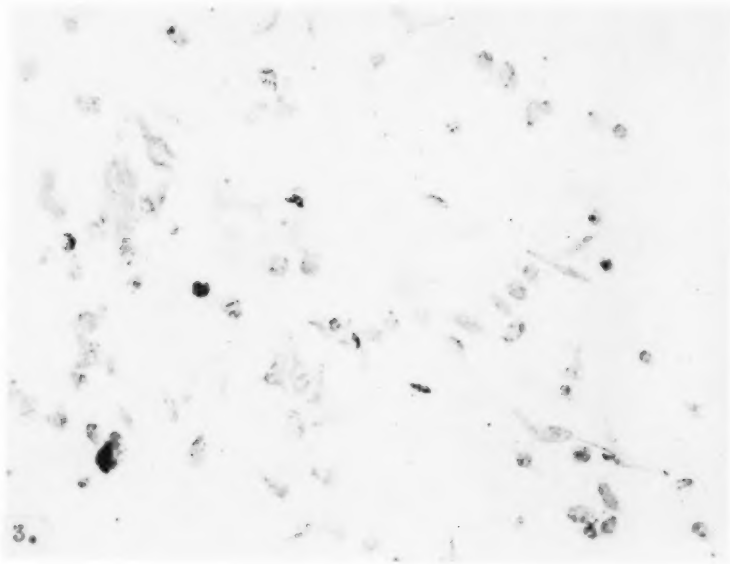


FIG. 1. Control cells on the fifth day, with a rare cell loaded with tubercle bacilli (*see* arrows). No appreciable injurious effect of other intracellular bacilli on cells is seen at this time even though 70 per cent contain bacilli, and later multiplication indicated that many of these were living cord-forming organisms.

FIG. 2. Intracellular cord formation without injurious effect on cell. Such multiplication never occurred in the extracellular area because of the streptomycin in the medium.



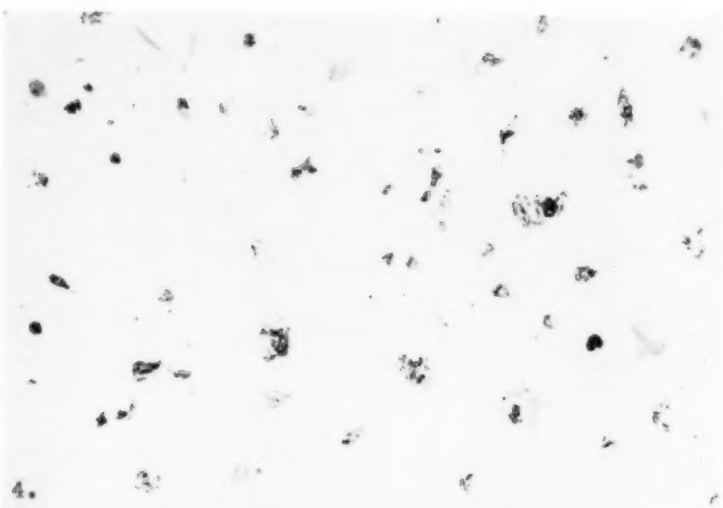


FIG. 3. Immune cells, originally heavily infected, on the seventh day. Note cord production and occasional rounded, loaded cell. No bursting cells are seen.

FIG. 4. Control cells on the seventh day, originally infected in an identical manner to those in figure 3. Marked evidence of intracellular multiplication, with most of the cells containing cords, or solid masses of bacilli and many bursting cells.

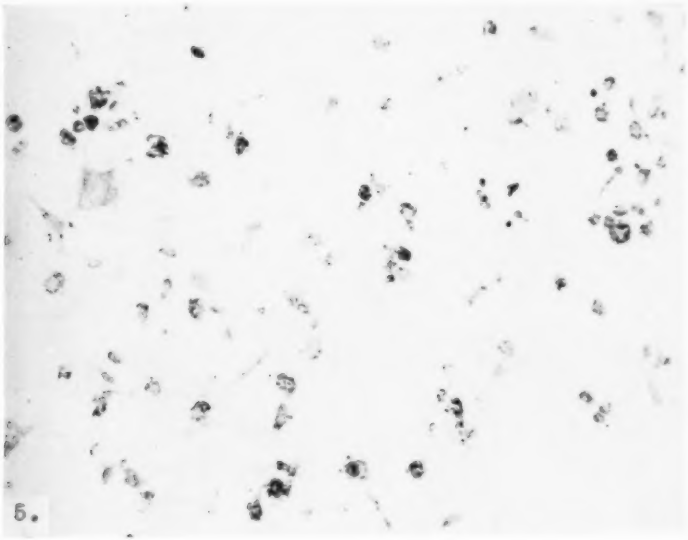


FIG. 5. More advanced multiplication of bacteria in immune cells at the twelfth day. Same experiment as figures 3, 4, and 6. Cell morphology is still good in some heavily infected cells, but rounded and bursting cells are numerous.

FIG. 6. Control cells on the twelfth day, now killed by intracellular multiplication of bacilli. Same experiment as figures 3, 4, and 5. Each bacterial colony is a site of cell death and disruption. Magnification is double that of figures 3, 4, and 5 to show colonial morphology When a cell dies there is some separation of cords, but multiplication ceases because of streptomycin in the medium.

side-wall cultures, or differences in vacuolation or other evidences of injury of the cells in the fixed and stained preparations.

Uninfected cell cultures of both groups consistently showed more prompt migration. These cells were usually smaller and developed longer, more delicate pseudopods. By the third day, infected cultures usually approached or equaled the activity and normal appearances of these uninfected cultures, although infected cells remained larger and slightly more rounded. Multinucleated cells developed in both infected and uninfected monocyte cultures.

Because of the differences in the rates of intracellular multiplication to be discussed, all experiments clearly showed an increased delayed lethal effect of virulent bacilli upon normal cells. This effect was not visible until the sixth to the twelfth days when the rate of burst and, therefore, killed cells was noticeably greater in the normal than in the immune cells (figures 3–6).

Rate of Multiplication of Virulent Tubercle Bacilli in "Immune" and Control Cells

By counting the numbers of cells which contained from 1 to 5, 6 to 10, 11 to 20, and more than 20 bacilli per cell on successive days, it was thought that the rates of intracellular multiplication could be estimated. Comparisons of such counts revealed that there may be a slightly less rapid increase in the numbers of bacilli in the immune cells than in the control (tables 1–7). These differences hardly seem significant, but they were consistent.

The above counts did not distinguish initially intracellular and multiplying bacilli from those bacilli which had not been phagocytized until after the experiment had been begun and the streptomycin was added to the medium. Moreover, the counts likewise did not identify the bacilli that had been released from a dying cell and which, therefore, had been rephagocytized

TABLE 1

BCG Immunization

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	55	20	0	0	41	15	0	0
2	43	26	1	0	49	11	1	0
4	27	41	25	2	43	36	4	0
5	25	47	25	0	29	46	16	0
6	35	31	22	0	17	41	41	10
7	23	37	37	1	23	49	23	15
8	33	30	21	1	12	35	51	30
11	11	40	40	54	22	38	34	15
12	6	50	44	20	34	45	5	0
13	30	45	11	0	37	41	11	0

TABLE 2

BCG Immunized and Desensitized

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	44	27	3	0	37	48	2	0
3	33	51	3	0	25	55	13	5
4	29	49	19	0	12	46	40	0
5	15	46	54	3	4	43	53	30
7	15	41	34	12	13	44	43	15
8	18	41	36	3	40	29	23	0

TABLE 3

BCG Immunized and Desensitized

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	61	4	0	0	57	5	0	0
2	61	9	3	0	65	7	0	0
3	53	25	8	0	52	35	7	0
4	28	52	9	0	58	21	12	2
6	30	50	15	15	15	25	58	13
7	50	20	17	16	35	25	38	23
8	39	37	19	18	*	*	*	37
9	*	*	*	18	20	27	51	32
12	2	46	51	38	*	*	*	200

* Counts technically impossible

TABLE 4

Air-borne Immunization

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	22	1	0	0	15	4	0	0
3	55	22	0	0	53	5	3	0
5	33	30	12	0	45	28	10	10
7	58	22	3	0	15	39	18	14
9	50	22	2	0	48	25	5	16
11	59	14	6	0	31	35	24	5

TABLE 5

AIR-BORNE IMMUNIZATION

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
0	11	9	2	0	24	3	0	0
1	26	20	4	0	33	19	1	0
3	44	18	0	0	34	24	13	0
5	25	26	7	0	21	35	17	30
6	58	9	1	0	14	55	20	10
8	8	61	31	0	17	70	13	16
9	8	60	28	0	3	42	53	21

TABLE 6

AIR-BORNE IMMUNIZATION

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	33	17	0	0	37	5	0	0
3	40	10	2	0	30	24	0	0
4	36	6	0	0	15	8	13	13
5	29	27	26	2	*	*	*	*
7	20	35	25	6	18	19	57	32
9	37	27	31	12	37	38	16	94
12	15	28	50	40	*	*	*	50

* Counts technically impossible

TABLE 7

AIR-BORNE IMMUNIZATION

Day	IMMUNE				NORMAL			
	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field	Number of Bacilli Per Cell			Burst Cells Per Low-Power Field
	1–5	6–20	21–50		1–5	6–20	21–50	
1	46	13	0	0	48	6	0	0
3	35	36	10	0	45	26	8	6
4	16	52	17	3	25	34	13	40
6	38	35	16	0	24	30	36	12
9	47	29	7	13	8	46	40	31
10	12	43	36	0	9	61	19	30
12	18	50	21	0	8	56	28	46

after exposure to streptomycin. Consequently, it was finally elected to count only those cells which contained bacilli that formed cords (figure 2) or compact intracellular masses (*see* figures 1, 3), or those that showed actual bursting of cells by the mass of intracellular bacilli (*see* figures 4–6). These were plainly visible with a low-power objective as dark red cords or masses in the cells (*see* figure 1).

These unequivocal evidences of intracellular multiplication were first observed about the fourth day of cultivation, although in other experiments they may not appear until the sixth day. On the fourth to sixth days with light infections these cells were rare and would not be encountered often enough in the counts of 100 to 200 cells to influence the rates of increase of intracellular bacilli. Furthermore, the actual numbers of bacilli in the clumps could not be enumerated because of the compactness of the masses. Even with heavy infections or late in the experiment (during the eighth to fourteenth days, when such cells became more frequent), these cells had to be listed separately from those cells in which exact counting of the numbers of intracellular bacilli was possible. Cells which contained cords, solid masses, or which were bursting with bacilli were, therefore, counted per low-power field. By the use of this method it was at once apparent that the immune cells consistently inhibited the intracellular multiplication of bacilli.

In those experiments in which the initial infection was light, the immune cells showed much less intracellular cord formation than did the control cells. Occasionally, no solid masses of bacilli or bursting cells were found in the immune cells, while at the same time many intracellular cord colonies and many cells packed with bacilli or bursting from bacilli were seen in the control cells (*see* tables 4, 5). In heavier infections the immune cells showed increased evidence of intracellular multiplication (*see* figures 3, 5) but the control cells (*see* figures 4, 6) of such experiments always demonstrated significantly more bursting cells (*see* tables 6, 7).

DISCUSSION

In tissue culture studies of cellular resistance to bacterial disease, particularly in a disease in which the first clinical or experimental effects of the infection *in vivo* may take months to become manifest, the determination of satisfactory end points is extremely difficult when the cells are studied for only approximately two weeks. The most commonly employed methods have been to measure the rate of cell death, the incidence of evidence of cell damage, or to count the increases in the numbers of intracellular bacilli as the experiment progresses, all in comparison with con-

trol cultures. When cells are being grown on glass, in clots, or free in the media, dead cells may be lost from the surfaces or may undergo lysis so that the first procedure may be unreliable. Enumeration of intracellular bacteria is also suspect since some extracellular bacilli are always present at the start of the experiment. In addition, those bacteria released from dying cells, no matter what the cause of death, will be rephagocytized by living cells. Tissue culture experiments with virulent tubercle bacilli require an inhibitory concentration of streptomycin in the medium which has been shown to be not inhibitory for intracellulary situated bacilli (7). Without this amount of streptomycin, the extracellular bacterial multiplication quickly overgrows the culture. Therefore, bacilli which are phagocytosed from the medium after the start of the experiment will have been exposed to inhibitory concentrations of streptomycin. The initial phagocytosis is carried on in a medium free of streptomycin. It appears from the cultures that significant numbers of monocytes which show increasing numbers of bacilli are the result of this rephagocytosis of drug-inhibited bacilli. Most of such intracellular bacilli are isolated single organisms; thus, they do not represent the consequences of multiplication. Cells with 20 or even 50 such bacilli are not injured by this type of infection regardless of how long the cells are observed.

The rate of intracellular multiplication will increase the rate of cell death when virulent bacilli are used. Rapid cell death will, therefore, result in the rapid release of bacilli into the medium for rephagocytosis. Therefore, rapid increases in intracellular bacilli, even if those bacilli may no longer multiply in the cell (possibly due to contact with the inhibitory concentrations of the streptomycin in the medium), may be indicative of the rate of initial intracellular bacillary multiplication. Thus, in comparing virulent with attenuated or avirulent bacilli, counts of intracellular bacilli on successive days of the experiment show extreme differences (6, 8). When dead bacilli are used, only very slight or no increases in intracellular bacilli occur (6, 8). On the other hand, the present experiments do not show large differences in countable numbers of intracellular bacilli between cells from immunized animals and cells from control animals similarly infected with virulent human tubercle bacilli. This correlates with the observation that no appreciable differences in the rate of cell death were noted in the two groups of cells.

The study has led to the belief that the only unequivocal evidence of intracellular multiplication is the appearance of intracellular cords and the gradual development of cells engorged, or literally bursting, with masses or colonies of intracellular bacilli (*see* figures 3, 4). In the medium which contains streptomycin in a concentration of 5 γ per ml., extracellular multiplication is completely inhibited. The original bacillary suspension was always filtered through a medium sintered glass filter. Microscopic examination of the bacterial suspension which was used to infect the cells never revealed significant clumps or cords of bacilli; the vast majority of organisms were single cells with a rare clump of 2 or 3 adherent bacilli. The development of the cords, large clumps, and bursting cells was gradual. Intracellular cords appeared first on the fourth or fifth day. The massive engorgement of the monocytes and finally the bursting of the cells occurred from the sixth day, progressively increasing until the conclusion of the experiment. It was only by counts of the relative frequence of these cells that significantly positive evidence was obtained which indicated that immune cells may have inhibited the intracellular multiplication of virulent tubercle bacilli to a greater degree than did normal cells.

This conclusion is in agreement with Suter's observations, although he based his results on the numerical counts of increasing intracellular bacilli. Suter also commenced his experiments with low incidences of infection; only 8 to 10 per cent of his cells were infected at the start. With these levels of infections, it has been more difficult to draw conclusions. The death of some of the infected cells early in the experiment, coupled with the natural mortality or loss of cells in the tissue culturing of monocytes in the present experiments, has led to such a reduction of numbers of cells infected with living bacilli by the seventh day of the experiment that intracellular multiplication may no longer be apparent or results numerically significant. In order to have significant numbers of cells with progressive intracellular multiplication in the later days of the experiment, an initial infection level of 30 to 50 per cent is preferred. In contrast to Suter's observations, if the number of infecting bacilli per cell is low, i.e., 1 to 3 bacilli per cell, excessive initial mortality

has not occurred and such cultures will appear essentially as healthy at three to five days as do the uninfected control cells. In all experiments, even cells which contain cords or small colonies of bacilli will appear normal until the intracellular mass becomes large, and often until the mass seems to burst the cell mechanically.

The present observations, as well as those of Suter, may be questioned with respect to hypersensitivity. Most of the immunized guinea pigs showed a 2- to 4-plus skin reaction to 10 mg. of intradermal OT. Because it was not possible, at the low bacillus-per-cell infection level, to detect differences in cell mortality or appearance which might be attributable to hypersensitivity to the intracellular tubercle bacilli, some cell injury may have occurred which might result in inhibition of intracellular multiplication by the streptomycin in the medium. In experiments using BCG-immunized guinea pigs desensitized until the skin tests were negative, however, some reduction in the rate of intracellular development of bacillary masses was also observed when compared with that observed in infected control cells. BCG immunization by the subcutaneous route would appear less effective in establishing the resistance than air-borne infection, so that the results using BCG-immunized animals are less convincing.

The failure of Mackaness to note the cellular resistance observed in the present studies may be the result of the very high infection level which he used. When infections of 5 to 10 bacilli per cell, as were routinely attained by Mackaness, were used either inadvertently or deliberately in the present experiments, the observed resistance might be overcome, as intracellular multiplication usually overwhelmed the cells from both immunized or normal animals during the first five days. Furthermore, the writers believe, as does Suter (9), that when such large numbers of bacilli are used, death or injury of cells may occur acutely because of the hypersensitivity reaction. This phenomenon will, therefore, tend to negate any effect of immunity in such experiments.

A comparison of these observations with those of Fong and his associates (5, 10) is difficult as they used a very high infection level of 10 bacilli per cell without streptomycin in the medium. Under these circumstances, in the present study, when virulent organisms were used, injurious and lethal effects on the monocytes rapidly occurred in an even more severe fashion than Fong observed. As they mentioned, avirulent organisms under identical conditions have no effect on the cells. Since they did not maintain their cultures for longer than three days, they naturally would not observe the cord formation or bursting cells which it is believed represent the most important evidence of intracellular multiplication. It is believed that the improved appearances which Fong noted at seventy-two hours may not be "cell proliferation" but rather the recuperation of injured, but not killed, cells. This improvement has been noted consistently in the present studies at three days in the absence of appreciable numbers of mitotic figures.

The protective action of immune serum on the initial cytotoxic effect of virulent bacilli has not been observed in the present experiments, although most of the observations have been concentrated on the cultures on and after the third day rather than at twenty-four and forty-eight hours. Observations on the effect of immune serum will be described in a future presentation.

The general observations in this study on the growth of tubercle bacilli in monocytes are remarkably similar to those made by Shepard in the HeLa cell (11). The same gradual appearance of bacterial cords and the later development of cells loaded or bursting with bacilli seen in monocytes were reported by him for HeLa cells. The same variations with bacterial virulence have also been noted. Shepard did not, however, describe an initial cytotoxic effect, best seen by the present writers twenty-four hours after infection. Furthermore, when streptomycin is not used, it has been impossible to be certain that intracellular cords which are seen during the latter days of the experiment are not the consequence of extracellular cord formation followed by phagocytosis. With 5 γ of streptomycin in the medium, this does not occur and all intracellular cords are indications of intracellular multiplication. Naturally, Shepard could not discuss the effects of immune cells on bacillary multiplication.

Summary

Tissue culture experiments which employed monocytes from guinea pigs immunized against infection with tubercle bacilli were performed. The monocytes were infected with virulent human tubercle bacilli and the *in vitro* infection was compared with that in monocytes from nor-

mal guinea pigs, both of which were maintained in normal guinea pig serum. No significant differences in rate of cell death and only minor changes in numbers of intracellular bacilli were noted between the two groups of cells. The only significant evidence of intracellular multiplication of tubercle bacilli was found to be the development of intracellular cords and the final appearance of masses of bacilli which filled and finally burst the monocyte. Monocytes from immune animals have an apparent ability to restrict the intracellular multiplication of virulent tubercle bacilli which leads to the development of intracellular cords, solid bacterial masses, or burst cells. This resistance was relative to control cells, not a complete immunity.

Sumario

Estudios en Cultivos de Tejidos sobre la Resistencia en la Tuberculosis. II. Monocitos de Cobayos Normales e Inmunizados Infectados con Bacilos Tuberculosos Humanos Virulentos

En estos experimentos con cultivos de tejidos se emplearon monocitos procedentes de cobayos inmunizados contra la infección por bacilos tuberculosos. Los monocitos fueron infectados con bacilos tuberculosos humanos virulentos y se comparó la infección *in vitro* con la de los monocitos procedentes de cobayos normales, manteniéndose ambos grupos en suero de cobayo normal. No se notaron mayores diferencias en la cifra de la mortalidad celular y apenas ligeros cambios en las cantidades de bacilos intracelulares entre los dos grupos de células. El único signo importante de multiplicación intracelular de los bacilos tuberculosos resultó ser la formación de cordones intracelulares y la aparición final de masas de bacilos que llenaron y por fin reventaron el monocito. Los monocitos procedentes de animales inmunes poseen una capacidad aparente para limitar la multiplicación intracelular de los bacilos tuberculosos virulentos, que conduce a la formación de cordones intracelulares, masas bacterianas sólidas o células reventadas. Esta resistencia fué relativa con las células testigos, no inmunidad total.

Resume

Etude sur la résistance des cultures tissulaires dans la tuberculose. II. Monocytes de cobayes normaux ou immunisés inoculés avec des bacilles tuberculeux humains virulents

On a procédé à l'expérimentation de cultures de tissus en utilisant des monocytes de cobayes immunisés contre l'infection par le bacille tuberculeux. Les monocytes étaient infectés par des bacilles tuberculeux humains virulents et l'infection *in vitro* était comparée avec celle provoquée par les monocytes de cobayes normaux; les uns et les autres étaient maintenus dans du sérum de cobayes normaux. Entre ces deux groupes de cellules, il n'a été observé aucune différence appréciable quant à la vitesse à laquelle se produisait la mort des cellules et aux variations mineures du nombre des bacilles intracellulaires. La seule manifestation évidente de multiplication intracellulaire du bacille tuberculeux qui ait été décelée, consistait en formation de cordes intracellulaires et, ultérieurement, dans l'apparition de masses de bacilles qui remplissaient le monocyte, qui finissait par éclater. Les monocytes de l'animal immunisé témoignaient de la capacité de restreindre la reproduction intracellulaire des bacilles tuberculeux virulents en provoquant successivement la formation de cordes, de masses bactériennes denses ou l'éclatement des cellules. Cette résistance était relative par comparaison avec les cellules témoins, mais ne représentait pas l'immunité complète.

Acknowledgment

The photomicrographs illustrating this paper were taken by Mr. Glenn Mills.

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Case Reports

GIANT LYMPH NODE HYPERPLASIA OF THE MEDIASTINUM[1]

Case Report with a Review of the Literature

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(Received for publication July 24, 1958)

Introduction

Malignant lymphoma, manifested as lymphosarcoma or Hodgkin's disease, is the most common neoplasm among mediastinal tumors. On the other hand, benign tumors of lymphoid origin, either inflammatory or neoplastic, are exceedingly rare. The purpose of this paper is to report a case of reactive lymph node hyperplasia of the mediastinum attaining an unusual size. Four cases collected from the Japanese literature are reviewed which the present writers believe fit into this condition. They also wish to call attention to this entity because no reports could be found in the literature since Castleman's first description of the condition in 1954.

Case Report

A 54-year-old female was referred to the surgical clinic because of an intrathoracic mass. She had been entirely healthy until one month prior to the present admission when she developed fever, general fatigue, cough, and left back pain radiating to the left upper arm. Her case was diagnosed as a common cold and was treated symptomatically, with slight improvement. However, general fatigue and left back pain persisted and a mass was discovered in her left chest on roentgenographic examination. Chest pain, hoarseness, expectoration, and dysphagia were not present.

Physical examination revealed a well-developed, moderately well-nourished, middle-aged woman who did not appear chronically ill. Some lymph nodes were noted in the left supraclavicular fossa, the largest being about the size of the tip of the thumb. On percussion, an area of dullness was found in the left upper anterior chest. Breath sounds over this area were diminished. No marked changes were noted in the back. Heart sounds were clear. There was no unusual abdominal condition.

Laboratory data showed the following: erythrocytes, 304 times 10^4; leukocytes, 6,000 per cu. mm.; the Schilling differential count was normal except for slight eosinophilia amounting to 7 per cent. The hemoglobin was 68 per cent (Sahli); the erythrocyte sedimentation rate was 120 mm. per hour (Westergren); the urinalysis was normal and the electrocardiogram was within normal limits.

The chest roentgenogram revealed a mass bulging from the superior mediastinum into the left upper lung field. Calcified nodules were discernible within the shadow of the mass. The trachea was markedly displaced to the right (figure 1). A lateral roentgenogram disclosed the mass to be in the anterior mediastinum (figure 2). Fluoroscopic examination showed no pulsation of the mass and well-functioning diaphragms. Bronchoscopy revealed a narrowed trachea apparently compressed from the left side with the left tracheal wall bulging into the lumen. There was marked stenosis of the left main bronchus at the level of the carina, and this also appeared compressed from the left posterior and anterior sides. Biopsy of the lymph node in the left supraclavicular area was noncontributory to the diagnosis of the intrathoracic mass.

A left thoracotomy was done through a posterolateral approach. No adhesions were found in the thoracic cavity, and a large mass covered with mediastinal pleura was found above the aortic arch extending from the anterior to the posterior mediastinum. The mass was firmly attached to the anterior chest wall and to the superior vena cava which was inadvertently torn during the process of dissection, resulting in considerable hemorrhage.

Macroscopic findings: The mass, measuring 16 by 9 by 5.5 cm., weighed 390 gm. and was well encapsulated with a thick capsule. The surface which protruded into the thoracic cavity was smooth, while the surface attached to the anterior chest wall was lobulated. On section, it was somewhat firm in consistency, parenchymatous, reddish-brown, with some calcification in its central portion (figure 3).

Microscopic findings: Microscopic sections revealed lymphoid tissue which consisted primarily of lymphocytes resting on a delicate fibrous reticulum network with marked capillary proliferation (figure 4). There were no typical lymph follicles with distinct germinal centers. However, small germinal centers containing aggregates of reticuloendothelial cells were prominent, some of them resembling Hassall's corpuscle (figure 5). Phagocytic activity could be identified in some of the germinal centers, while others contained degenerated hyaline masses. There were focal infiltrations between the follicles of plasma cells and scattered eosinophils throughout the tissue. However, no Sternberg-Reed cells were identified.

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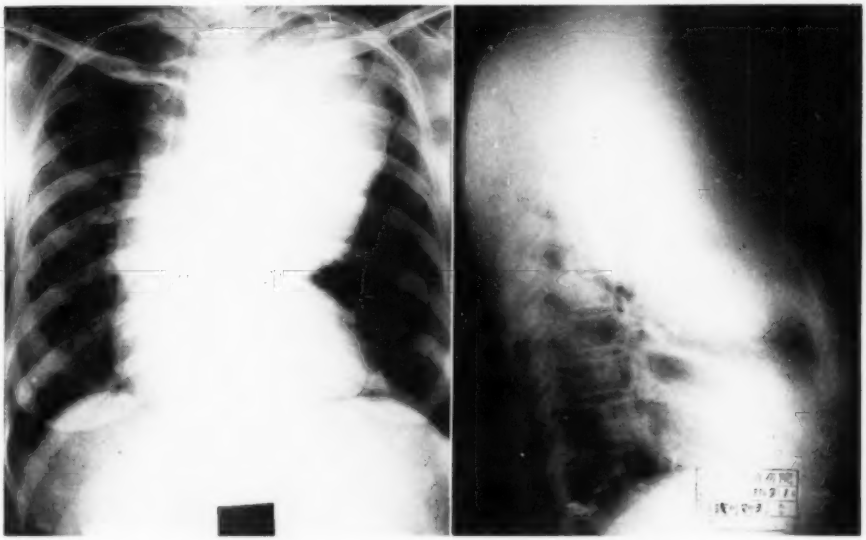

FIG. 1 (Left). Chest roentgenogram showing a large mass in the left upper lung field.
FIG. 2 (Right). Left lateral chest roentgenogram showing a mass to be located in the anterior mediastinum.

A pathologic diagnosis of reactive lymphoid hyperplasia was believed to be most likely.[2]

The postoperative course was uneventful and the patient was discharged from the hospital on the twenty-third postoperative day with the left lung well expanded.

DISCUSSION

Localized lymph node hyperplasia is an entity which Castleman separated and considered distinct from thymoma (1, 2). Superficially, the lesion has some resemblance to thymoma, which may cause one to be mistaken for the other, which can be unfortunate, as emphasized by Castleman. He pointed out several characteristic features of lymph node hyperplasia. Clinically, most of them develop in the mid mediastinum around the hilum, closely adjacent to the tracheobronchial tree. Microscopically, there are two distinct features: hyperplasia of lymphoid follicles and marked capillary proliferation with endothelial hyperplasia.

It is very rare that the lymph nodes attain a size larger than a hen's egg in hyperplasia. Ackerman (3), states that he has encountered

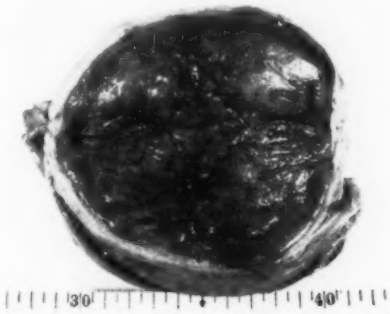

FIG. 3. Cut surface of the mass showing relatively thick capsule and homogenous smooth appearance.

hyperplastic nodes as large as 5 cm. in the mediastinum and 8 cm. in the inguinal region. It is only natural to doubt the lymphoid origin of the present case because of its unusual size and location. Castleman (2), however, had a case in his series of 13 cases in which the tumor measured 15 by 11.5 by 9 cm. and was located in the right anterior mediastinum.

It is surprising that many writers reporting

[2] Dr. B. Castleman of Massachusetts General Hospital, Boston, studied the sections and concurred in the diagnosis.

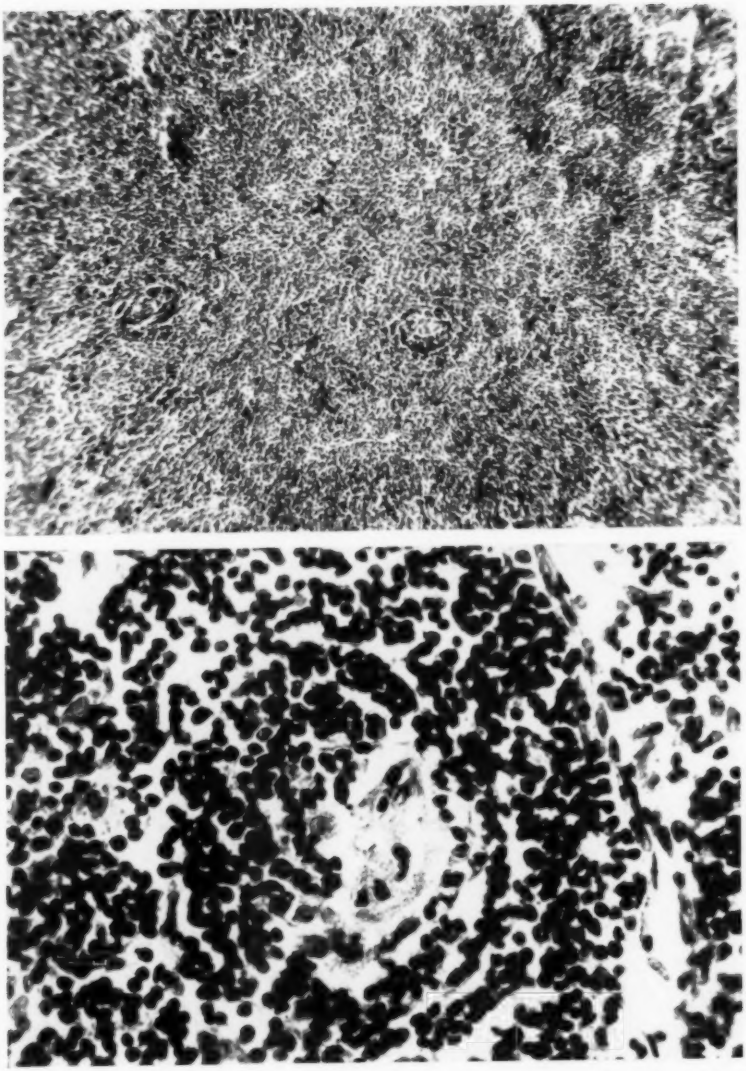

FIG. 4 (Upper). Microscopic section showing lymphoid tissue consisting primarily of lymphocytes. (Hematoxylin-eosin stain, × 50)

FIG. 5 (Lower). Microscopic section showing germinal center with peripheral cuff of lymphocytes giving some resemblance to Hassall corpuscle. (Hematoxylin-eosin stain, × 400)

large numbers of cases of mediastinal tumors have not mentioned this condition, although Castleman has seen about 25 cases belonging in this category (4).

Review of Literature

The present writers were able to collect 4 cases from the Japanese literature which can be considered to fit into the category of lymph

TABLE 1
FIVE CASES OF LYMPH NODE HYPERPLASIA OF THE MEDIASTINUM

Case Number Authors	Age Sex	Initial Complaint	Duration of Mass	Size	Location
			years		
1 Inada *et al.*	18 M	Fatigue	2	3 by 4 by 6.5 cm.	Left hilum
2 Kurobane *et al.*	14 F	Roentgenographic finding	—	Hen's-egg size	Right hilum interlobar
3 Wada *et al.*	24 F	"Colds"	—	Apple size	Right anterior inferior mediastinum
4 Nakano *et al.*	30 F	Roentgenographic finding	5	Small fist and walnut size	Left hilum
5 Inada *et al.*	54 F	"Colds"	—	16 by 9 by 5.5 cm.	Left anterior superior mediastinum

node hyperplasia (table 1) (5–8). One of the cases had previously been erroneously reported as aberrant thymoma as had two of the thirteen cases of Castleman's series. One case was diagnosed as nonspecific lymphadenitis. It is conceivable that there might have been more cases which were mistaken for other diseases or were unreported because of unknown genesis.

The ages of the patients in the 4 cases from the Japanese literature and in the present one ranged from fourteen to fifty-four years. There were 4 females and one male patient, although in Castleman's series there was no sex predominance. Symptoms referable to the mass were absent in half of the cases and discovery of the mass was accidental or incidental, as in Castleman's series. It is noteworthy that there were no symptoms referable to the mass despite the presence of marked stenosis of the left main bronchus in the reported cases.

In *Case No. 4* (table 1), the presence of the mass had been recognized for five years without any changes being apparent roentgenographically. In Castleman's series, the longest period in which a mass was apparent was eight years.

It is significant that the masses were larger than a hen's egg, suggesting gradual enlargement over a long period of time. Focal calcification as noted in 2 of the cases (the present case and *Case No. 1*) also support this view. According to Castleman, the lesion usually involves only one lymph node. In *Case No. 4* there were two lymph nodes involved, one about the size of a small fist and the other about the size of a walnut, both of which presented the same histologic picture.

It is interesting to note that, histologically, no typical lymphoid follicles with prominent germinal centers were found in the 2 cases of focal calcification (the present case and *Case No. 1*), while they were prominent features in the other 3 cases (figures 6 and 7). Abundant plasma cells with Russell bodies were present between follicles in *Case No. 4*. Such a case has been mentioned by Castleman in his series. Under such circumstances, it is important to differentiate plasma cell tumor as described by Childress and Adie (9) or Spyker and Kay (10).

It would be superfluous to state here that these cases should be treated surgically as soon as possible after detection. However, overzealous surgical procedures should be avoided. Castleman described two cases in which lobectomy and pneumonectomy were performed because the masses were mistaken for bronchial adenoma. In *Case No. 1*, it was necessary to excise the mass with the left upper lobe because of the presence of firm adhesions.

SUMMARY

A case has been reported of localized lymph node hyperplasia in the mediastinum which reached an unusual size. A 54-year-old female complaining of fatigue and left back pain of one month's duration was discovered on roent-

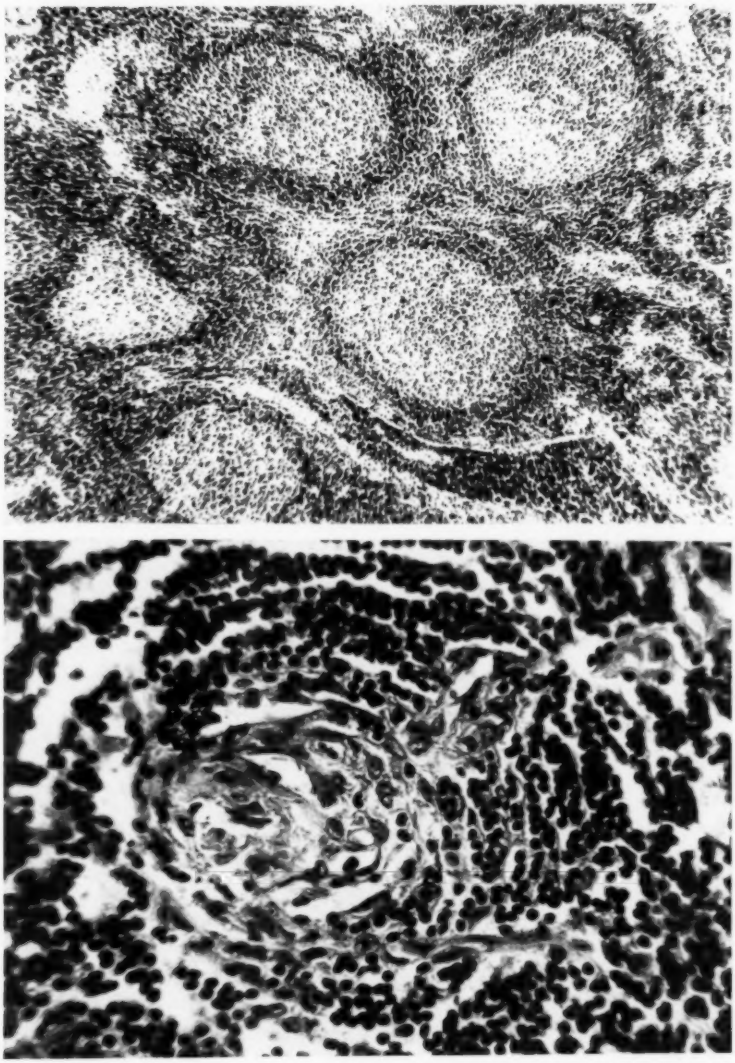

FIG. 6. *Case 4* (Upper). Microscopic section showing hyperplasia of lymphoid follicles with characteristic germinal centers. (Hematoxylin-eosin stain, × 50)

FIG. 7. *Case 3* (Lower). Microscopic section showing partially hyalinized follicle with sprouting vessels. (Hematoxylin-eosin stain, × 300)

genographic examination to have a mass in the anterior mediastinum. On left thoracotomy, a mass measuring 16 by 9.5 by 6 cm. was successfully removed. Microscopic sections revealed reactive lymphoid hyperplasia resembling a thymoma. A collective review of four cases in the Japanese literature was made, and the significance of lymph node hyperplasia in the mediastinum as a separate clinical entity was emphasized.

Sumario

Hiperplasia Gigante de Ganglio Linfático del Mediastino, Presentación de un Caso con Repaso de la Literatura

Preséntase un caso de hiperplasia localizada de un ganglio linfático en el mediastino, que alcanzó tamaño inusitado. En una mujer de 54 años de edad que se quejaba de fatiga y raquialgia del lado izquierdo desde un mes antes se descubrió en el examen roentgenográfico una tumefacción en el mediastino anterior. En una toracotomía izquierda, se extirpó con éxito una tumoración que media 16 por 9.5 por 6 cm. Los cortes microscópicos revelaron hiperplasia linfoidea reactiva parecida a timoma. Se hizo un repaso colectivo de 5 casos de la literatura japonesa y se recalca la importancia de la hiperplasia de los ganglios linfáticos como entidad clínica independiente.

Resume

Ganglion lymphatique géant du médiastin. Observation d'un cas et compte-rendu de la littérature médicale

Présentation d'un cas d'hyperplasie d'un ganglion lymphatique localisé dans le médiastin et qui avait atteint des dimensions exceptionnelles. Chez une femme de 54 ans se plaignant depuis un mois de fatigue et d'une douleur dorsale située du côté gauche on a découvert à l'examen radiologique une masse située dans le médiastin antérieur. Une thoracotomie gauche a permis d'enlever avec succès une masse mesurant 16 cm x 9.5 cm x 6 cm. Les coupes histologiques révélèrent une hyperplasie lymphoïde réactionnelle dont l'aspect était celui d'un thymome. L'analyse de cinq cas relevés dans des publications japonaises a été effectuée en collaboration par les auteurs et la valeur de l'hyperplasie ganglionnaire comme entité clinique séparée a été soulignée.

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MEDIASTINAL TUBERCULOSIS MANIFESTED BY PERICARDITIS, OSTEOCHONDRITIS, AND BRONCHOESOPHAGEAL FISTULA[1]

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Introduction

The protean manifestations of tuberculosis are well known, and it has been said that no organ or tissue of the human body is immune from attack by tubercle bacilli (1). The case to be described presented an unusual triad, namely, tuberculous involvement of the pericardium and first rib in association with a bronchoesophageal fistula.

Case Report

O. F. (NHH 47-32-55), a 29-year-old Negro female, was admitted to the hospital on August 2, 1957, because of fever, cough, and dysphagia. She had been well until four months prior to admission, when she noted a persistent, nonproductive cough. One month later she developed intermittent rigors and became tired, weak, and increasingly dyspneic. Three weeks before admission, fever and night sweats appeared. At the same time, the patient noted burning substernal pain accentuated by coughing. The symptoms progressed and one week before admission the cough became productive of copious amounts of white, mucoid sputum. She found that particles of food "stuck in her throat" and that swallowing of food or even saliva initiated violent paroxysms of coughing. She became progressively weak and anorectic and lost 40 pounds in the course of the illness.

Past history: The patient was born in South Carolina, and had lived in New Haven, Connecticut, for five years. A cousin was admitted to another hospital because of tuberculosis, but the patient had not been in close contact with him. Three years before admission she had had a right salpingo-oophorectomy.

Physical examination: Temperature was 104°F., the pulse 140 per minute, respirations 40, and the blood pressure was 90 systolic, 70 diastolic in mm. of mercury. The patient was an obese Negro female who appeared most comfortable when lying on her right side; she coughed frequently during the examination. The skin was hot and dry. A prominent subconjunctival hemorrhage was present on the left. A tender, firm swelling, 4 cm. in diameter, was noted over the anterior aspect of the right first rib. The lungs were clear to percussion and auscultation. The heart was not enlarged. A loud pericardial friction rub and a soft precordial systolic murmur were audible at the left sternal border in the third and fourth intercostal spaces. Venous distention was absent and no paradoxical pulse was noted. The liver was percussed 2 fingerbreadths below the right costal margin and the spleen and kidneys were not palpable. Pelvic examination was unremarkable. Neurologic examination was normal. The patient was able to swallow water only with considerable difficulty, and paroxysms of coughing were precipitated by swallowing. Mirror examination of the larynx revealed adequate function of both vocal cords.

Laboratory data: The hematocrit was 37 per cent, hemoglobin 10.5 gm. per 100 ml., and the leukocyte count was 8,500 per cu. mm. with a normal differential. Urinalysis demonstrated a specific gravity of 1.030, 1-plus proteinuria, and a normal sediment. Stool guaiac was negative. Nonprotein nitrogen, fasting blood sugar, and serum electrolytes were normal. Blood serum protein was 6.19 gm. per 100 ml.; albumin 2.7 gm. per 100 ml.; globulin, 4.02 gm. per 100 ml. Antistreptolysin O titer, streptococcal agglutinins, and C-reactive protein were within the normal range. A serologic test for syphilis (VDRL) was nonreactive. A sickle-cell preparation was negative and no lupus erythematosus cells were found. The cerbrospinal fluid was entirely normal. Examination of sputum for malignant cells was negative on three occasions as was cytology on secretions obtained at bronchoscopy. Nose culture revealed a moderately heavy growth of hemolytic *Staphylococcus aureus*. Throat and sputum cultures showed normal flora. Three blood cultures were sterile. Administration of 0.00002 mg. of PPD intradermally yielded a positive reaction. Histoplasmin, blastomycin, and coccidioidin skin tests were negative. Tubercle bacilli were not demonstrated in stained films but were cultured from the sputum on three occasions.

The venous pressure was 130 mm. saline; circulation time was normal; and an electrocardiogram revealed a sinus tachycardia, RS-T segment depression in leads I, II, and V2-V6, diphasic and inverted T waves in I, II, AVL, AVF, and V4-V6. This was interpreted as consistent with pericarditis.

Roentgenograms: A roentgenogram of the chest revealed a left pleural effusion and a large globular

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FIG. 1 (Left). Chest roentgenogram prior to operation showing globular heart and left pleural effusion.

FIG. 2 (Center). Roentgenogram of the heart with patient in left lateral decubitus following CO_2 injection intravenously. Gas outlines right border of the heart showing increased distance between pericardium and epicardium.

FIG. 3 (Right). Roentgenogram of the esophagus revealing contrast material passing from the esophagus into the left main stem bronchus.

FIG. 4 (Left). Section of biopsy of the first rib (×150). Caseation and giant cells of Langhans' type have completely replaced bone and cartilage.

FIG. 5 (Center). Section of pericardium (×125). Fibrinous pericarditis is present as well as caseation and giant cell formation.

FIG. 6 (Right). Section of mediastinal lymph node (×125) showing distortion of architecture by caseous necrosis.

Fig. 7. Chest roentgenogram six months postoperatively. There is residual right pleural thickening.

heart (figure 1). Fluoroscopy demonstrated diminished pulsation of the diaphragmatic aspect of the heart with the patient erect, and widening of the superior mediastinum in the prone and supine positions. Injection of 50 ml. of carbon dioxide into the left antecubital vein (2) showed that the right border of the heart and pericardium was thickened, a finding compatible with pericardial effusion (figure 2). Pericardiocentesis was attempted by the subxyphoid approach, and 5.0 ml. of grossly bloody fluid were removed. The fluid was sterile and had a hematocrit of 27 per cent. Thoracentesis yielded 10 ml. of yellow turbid fluid which contained numerous mononuclear cells, but smear and culture failed to reveal acid-fast bacilli or bacteria.

A roentgenogram of the esophagus demonstrated a communication between that organ and the left main stem bronchus (figure 3). Esophagoscopy confirmed the presence of this fistula 27 cm. from the incisors on the anterior esophageal wall. There was no evidence of traction on the esophagus. At bronchoscopy the fistulous opening was seen to lie 2.5 cm. beyond the carina in the medial wall of the left main stem bronchus. A biopsy of the bronchus at the fistulous opening showed squamous metaplasia and chronic inflammation.

Course in the hospital: Treatment was begun with 300 mg. of isoniazid, 12 gm. of para-aminosalicylic acid, and 1 gm. of streptomycin per day. Procaine penicillin, 600,000 units intramuscularly, was added to the regimen because of the suspicion of a superimposed nontuberculous bacterial pneumonia. During this period an infiltrate developed in the right lower lobe, presumably secondary to aspiration of food or fluids.

The fistula was approached surgically through a right thoracotomy incision with removal of the fifth rib. The fistulous tract between the esophagus and the left main stem bronchus was isolated, ligated, divided, and oversewn. The small hole in the esophagus was closed. Biopsy of the first rib, pericardium, and mediastinal lymph node revealed multiple granulomas with giant cells of the Langhans' type in all specimens as well as fibrinous pericarditis (figures 4–6). Acid-fast, Gridley, and Gomori silver stains failed to demonstrate fungi or tubercle bacilli in any of the specimens.

The patient became afebrile one week postoperatively, and therapy with isoniazid and para-aminosalicylic acid was continued. She was transferred to a sanatorium for convalescence and, when seen six months postoperatively (at which time she was still on antituberculous medication), she felt well. A roentgenogram of the chest at that time demonstrated only right pleural thickening with clear lung fields and a heart of normal size (figure 7).

Comment

Fistulous communications between the trachea or bronchi and the esophagus are most often a consequence of a malignant process in that region (3, 4). Benign respiratory-esophageal fistulas are quite rare. A review of the world literature in 1949 revealed only seventy-five cases, fourteen of which were assumed to be tuberculous in origin (5). The clinical and pathologic findings in twelve of these patients are summarized in table 1 (4–10).

The pathogenesis of this lesion is of considerable interest. Heddaeus postulated in 1889 that tuberculous respiratory-esophageal fistulas resulted from erosion of caseous parabronchial lymph nodes into the contiguously located esophagus and tracheobronchial structures (11). There is little doubt that it took place in this patient in view of histologic proof that the mediastinal lymph nodes were involved by the tuberculous process. This was also the case in seven of twelve patients previously reported, who had definite evidence of tuberculosis of the mediastinal lymph nodes. Furthermore, it seems likely that in this patient and also in the one described by Westergren and Ragnell (6), the infection spread along the mediastinal lymphatics to involve the pericardium and rib. While it may be argued that the tubercle bacilli were disseminated by the hematogenous route, the lack of systemic symptoms, benign character of the course, and prompt response to treatment mitigate against this possibility. Nor can the lack of a roentgenographically demonstrable pulmonary focus be

TABLE 1

REPORTED CASES OF TUBERCULOUS ESOPHAGO-RESPIRATORY FISTULAS

Writer, Year	Type of Fistula	Associated Tuberculous Lesions	Comment
1. Westergren and Ragnell, 1927 (6)*	Broncho-esophageal	Pulmonary, pericardial, mediastinal lymphatic	Post-mortem examination
2. Glynn, 1933 (7)	Tracheo-esophageal	Not stated	Biopsy showed "tuberculous inflammation"; living, with patent fistula, follow-up study less than one year.
3. Monserrat, 1940 (8)	Broncho-esophageal	Pulmonary, hepatic, splenic, renal, intestinal, meningeal, mediastinal lymphatic	Post-mortem examination
4. Monserrat, 1940 (8)	Tracheo-esophageal	Pulmonary, peritoneal, mediastinal lymphatic, splenic	Post-mortem examination
5. Monserrat, 1940 (8)	Broncho-esophageal	Pulmonary, hepatic, mediastinal lymphatic, splenic	Post-mortem examination
6. Monserrat and Mosquera, 1942 (9)	Tracheo-esophageal	Mediastinal lymphatic, pulmonary, intestinal	Post-mortem examination
7. Moersch and Tinney, 1944 (10)	Not stated	Not stated	Post-mortem examination, sputum positive for tubercle bacilli
8. Moersch and Tinney, 1944 (10)	Not stated	Not stated	Sputum positive for tubercle bacilli
9. Moersch and Tinney, 1944 (10)	Not stated	Not stated	Sputum positive for tubercle bacilli
10. Moersch and Tinney, 1944 (10)	Not stated	Not stated	Sputum positive for tubercle bacilli
11. Clagett, Payne, and Moersch, 1946 (4)	Broncho-esophageal	Calcified nodes	Recovered after surgery; pathology, "chronic inflammation and tuberculosis."
12. Coleman and Bunch, 1950 (5)	Broncho-esophageal	Pulmonary, calcified mediastinal nodes	Recovered after surgery; pathology, no tubercles in fistula.
13. Shope and Petersdorf, 1958	Broncho-esophageal	Pulmonary, pericardial, bony, mediastinal lymphatic	Recovered after surgery; sputum positive for tubercle bacilli; pathology, tuberculosis of the rib, pericardium, and mediastinal node.

* Numbers in parentheses refer to references in present article.

cited as evidence favoring hematogenous dissemination, for active pulmonary tuberculosis may have been present at the onset when tubercle bacilli were cultured from the sputum.

This does not mean, of course, that tuberculosis beginning in the mediastinal structures cannot become widespread. In four cases examined at autopsy and reported by Monserrat, in addition to pulmonary and mediastinal tuberculosis there was involvement of the spleen, and also of the liver, peritoneum, kidney, meninges, and intestines in three of these cases (8, 9). Perhaps a needle biopsy of the liver in the patient reported herein would also have demonstrated granulomatous lesions. On the other hand, it seems more logical to postulate that involvement of the tracheobronchial tree, paratracheal lymph nodes, pericardium, and rib were all a manifestation of spread along mediastinal lymphatics, and that hematogenous dissemination is a late consequence of uncontrolled disease in the lungs and mediastinum.

The symptoms of pericarditis exhibited by the patient were classic and conform closely to those described by Harvey and Whitehill (12) in twenty cases of pericarditis with effusion. In their series, 25 per cent of the patients had chest pain, 85 per cent were dyspneic on exertion, and a pericardial rub was noted in 30 per cent. The pericardial effusion was bloody in 70 per cent and was associated with fluid in the pleural cavity in one-fourth of the patients. All of these findings were present in the patient reported herein.

Costovertebral tuberculosis, a common site of rib involvement, is almost always associated with paravertebral abscess. In only three of twenty-three cases reported by Tatelman and Drouillard (13) was the lesion confined to the anterior rib. It is of interest that these three patients did not have tuberculosis of the vertebrae, and it is conceivable that the mediastinal lymph nodes served as the infecting focus.

Summary

A case is reported of mediastinal tuberculosis involving the pericardium and ribs and presenting with broncho-esophageal fistula. It is postulated that this lesion was caused by erosion of a caseous parabronchial lymph node into the esophagus and bronchus, and that spread to the pericardium and rib occurred via the mediastinal lymphatics. Repair of the fistula and treatment with isoniazid and para-aminosalicylic acid resulted in recovery.

Sumario

Tuberculosis Mediastínica Traducida por Pericarditis, Osteocondritis, y Fístula Broncoesofágica

Preséntase un caso de tuberculosis del mediastino que afectó el pericardio y las costillas y produjo fístula broncoesofágica. Presupónese que esta lesión fué ocasionada por la erosión de un ganglio linfático parabronquial caseoso al esófago y al bronquio y que la difusión al pericardio y a la costilla tuvo lugar a través de los linfáticos del mediastino. La reparación de la fístula y el tratamiento con isoniacida y ácido para-aminosalicílico lograron la curación.

Resume

Tuberculose médiastinale se manifestant par une péricardite, une ostéochondrite et une fistule broncho-oesophagienne

Observation d'un cas de tuberculose médiastinale intéressant le péricarde et les côtes avec fistule broncho-oesophagienne. Les auteurs supposent que cette lésion a été déterminée par l'érosion d'un ganglion lymphatique caséeux avec drainage dans l'oesophage et la bronche, et dissémination dans le péricarde et la côte par les lymphatiques du médiastin. La fermeture de la fistule et le traitement par l'isoniazide et l'acide para-aminosalicylique ont entraîné la guérison.

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Notes

CIRCULATING LEVELS OF THE "PLASMA FACTOR" RESPONSIBLE FOR IN VITRO LEUKOCYTE CYTOLYSIS DURING SENSITIZATION OF GUINEA PIGS WITH MYCOBACTERIUM TUBERCULOSIS[1]

Favour[2] has shown that peripheral blood leukocytes from the tuberculin-hypersensitive host are lysed *in vitro* in the presence of tuberculoprotein. Miller and associates[3] later demonstrated that this *in vitro* cytolysis could be inhibited by adequate washing of the sensitive leukocytes before exposure to tuberculin. They further noted that washed cells or normal cells, when resuspended in tuberculous plasma, were lysed in the presence of tuberculoprotein. This observation led to the demonstration that a "plasma factor" was responsible for *in vitro* cytolysis.[4] It was the purpose of the present study to measure the plasma factor in the circulation of guinea pigs during the course of sensitization with *Mycobacterium tuberculosis* and to determine its relation to the appearance of skin sensitivity.

Forty male albino guinea pigs weighing approximately 300 gm. were used in this study. Thirty-six were injected intramuscularly with 1 ml. of sterile paraffin oil containing 5 mg. of heat-killed, human tubercle bacilli (H37Rv). The remaining 4 animals served as controls and received 1 ml. of sterile paraffin oil intramuscularly. The procedure for determining the presence of plasma factor was similar to that described by Miller and Favour.[5] Normal leukocytes were harvested from the whole blood of fasting normal guinea pigs. The animals were bled by cardiac puncture, using heparin as an anticoagulant. The pooled blood was centrifuged at 3,000 r.p.m. (1,500 g.) for twenty minutes at 4°C., and the buffy coats and plasma were carefully removed and recentrifuged. The resulting cell butt was resuspended in homologous plasma to one-fifth of the original blood volume and the final leukocyte concentration was adjusted to 25,000 per mm.^{3} by dilution with plasma.

Immediately prior to injection with the heat-killed bacilli and at regular intervals thereafter, fasting experimental animals were bled in groups of 4. The individual specimens of blood were allowed to clot at 4°C. and the sera were harvested after centrifugation in the cold. Because of the lability of the plasma factor,[4] cytolysis tests were done within four hours of bleeding. The control group of 4 animals was bled just prior to injection and at weekly intervals thereafter.

Cytolysis tests were performed as follows: for each test serum, two siliconized serologic test tubes (12 by 75 mm.) were used; each received 0.1 ml. of the buffy coat suspension and 0.3 ml. of the test serum; to one tube of each pair was added 0.1 ml. of saline containing 5 γ of PPD (Sharp and Dohme), and the other received 0.1 ml. of saline. The contents of the tubes were mixed well and samples were immediately withdrawn for total leukocyte counts. The tubes were then sealed with silicone stoppers and rotated for two hours at 37°C. on a roller drum revolving at 5 r.p.m., after which a second leukocyte count was made. Both chambers of a standard hemocytometer (AO Spencer) were counted. The same pipette and hemocytometer were used for the initial and final counts of each sample. The PPD decrement was calculated as the per cent change in the tuberculin tube minus the per cent change in the saline tube.

The animals in the experimental group were also tested intradermally with 5 γ of PPD during the sensitization period. The skin tests were made on groups of 4 animals on the seventh day following bleeding. The diameter of induration of the skin reactions was measured and recorded at forty-eight hours.

The results of the cytolysis tests and skin tests are presented in table 1. Each control and experimental value represents the mean of 4 animals. Previous workers[6, 7] have accepted a PPD decrement which exceeded −10.0 as significant. On this basis, significant decrements were found in the experimental group during the period from five to twelve days following sensitization. The decrements at these intervals were also statisti-

[1] The material presented will form part of a thesis to be submitted to George Washington University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE 1

CIRCULATING "PLASMA FACTOR" AND TUBERCULIN SKIN SENSITIVITY IN GUINEA PIGS AT VARIOUS INTERVALS AFTER INJECTION OF HEAT-KILLED TUBERCLE BACILLI IN PARAFFIN OIL

Interval	Control Group	Experimental Group	
	Mean PPD Decrement	Mean PPD Decrement	Mean Skin Reaction*
(*days*)			(*mm.*)
0	+0.2	+0.6	
3		−1.0	
5		−10.8	
7	−4.7	−14.6	5.5
10		−13.7	8.5
12		−14.8	9.0
14	+5.5	−3.4	11.0
17		+1.2	12.0
21	−5.5	+3.2	12.5

* Mean diameter of induration forty-eight hours after skin testing

cally different ($P < 0.05$) from the mean PPD decrement of the 0 day values. The control animals showed no significant decrements.

The marked PPD decrements observed from five to twelve days following sensitization indicate the presence, during this brief period, of the plasma factor. The early appearance and short duration of this factor in the circulation agrees with the results of studies by Waksman[7] in which a different technique was used. He mixed tuberculin with samples of whole blood collected from guinea pigs at various intervals after injection with heat-killed bacilli in mineral oil and found marked cytolysis on the thirteenth and fifteenth days following sensitization, but none on the nineteenth day. He also noted that cytolysis occurred before skin sensitivity was maximal, and was absent at a time when marked skin reactions were evident.

The results of this study also indicate that the plasma factor is present in the circulation before maximal skin sensitivity is manifested (*see* table 1). This point is of interest in relation to the recent work of Hall and Sherago[8] who demonstrated that leukocytic sensitivity to tuberculin, as measured by tissue culture methods, also develops earlier than skin sensitivity. O'Neill and Favour's observation[9] that humoral factors present in plasma may mediate tissue culture sensitivity adds significance to this finding. Studies are now in progress in this laboratory in an attempt to further elucidate the relationship of the cytolysis phenomenon and the plasma factor to tuberculin hypersensitivity.

In summary: Under the experimental conditions described, the "plasma factor" responsible for *in vitro* cytolysis of leukocytes by tuberculin has been found to appear in guinea pig serum early in the course of sensitization with heat-killed tubercle bacilli, before maximal skin sensitivity is manifested, and to persist for only a short time.

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EXPERIMENTAL REINFECTION IN ARRESTED GUINEA PIG TUBERCULOSIS AND ITS BEHAVIOR UNDER ISONIAZID THERAPY

It is well established that under appropriate conditions, experimental reinfection of tuberculous or BCG-vaccinated animals with virulent tubercle bacilli is unlikely to lead to progressive disease. Recently, experiments have been published dealing with the influence of reinfection on tuberculous animals which were undergoing drug treatment.[1-6] In some of these experiments reinfection did not occur, probably because the relatively small inoculum used for the challenge did not overcome the immune state caused by the primary infection. In others, although reinfection obviously progressed, the effects were not clear, since the results were expressed only as a function of the survival time of the animals.

The present note deals with the effect of reinfection on a primary tuberculosis arrested with isoniazid. A large inoculum has been employed in an attempt to overcome the protective effect of the first infection. It was the purpose to determine under these conditions: (*1*) if spreading really occurs, (*2*) whether the primary infection is reactivated or not, and (*3*) what is the effect of isoniazid when administered to reinfected guinea pigs. Drug-resistant mutants of virulent tubercle bacilli were used in these experiments, thus making it possible to follow the presence and degree of bacterial dissemination.[7]

Tuberculin-negative guinea pigs weighing 350 to 450 gm. were used; they were housed separately. The primary infective dose was always inoculated subcutaneously into the anterior aspect of the left thigh. Reinfection inoculum was injected subcutaneously in the anterior aspect of the right thigh. Inoculum size was 0.2 mg., wet weight, of bacilli. The bacterial suspensions were prepared with resistant mutants of the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*[8] grown for twenty-one days in Löwenstein-Jensen medium. The bacterial mass was determined in Hopkins tubes.

The streptomycin-[9] and viomycin-resistant mutants were obtained by serial transfer onto the surface of Löwenstein-Jensen medium containing increasing amounts of the drugs. These strains have been maintained in this laboratory for almost two years by periodic subculture in a drug-containing medium. The streptomycin-resistant strain was resistant to 1,000 γ of streptomycin per ml. and was found to be susceptible to 7.0 γ of viomycin per ml. The viomycin-resistant strain was resistant to 100 γ of viomycin per ml. and was found to be susceptible to 5.0 γ of streptomycin per ml. Both strains were susceptible to 0.1 γ of isoniazid per ml. Lower concentrations of the drugs were not tested. Each strain grew equally well on Löwenstein-Jensen medium with or without the appropriate drug.

When isoniazid was used, it was administered orally (5 mg. per day per guinea pig).

After infection and reinfection each guinea pig was continuously observed for the development of lesions and Koch's[10] phenomenon at the sites of infection and reinfection, respectively. Hypersensitivity tests using a tenfold dilution of Old Tuberculin were made monthly on all animals.

All of the guinea pigs were examined at autopsy, and touch smears, cultures in Löwenstein-Jensen, and hematoxylin-eosin sections of sites of infection, lymph nodes, spleen, liver, and lungs were made. Drug-susceptibility studies were performed on all of the recovered strains using Löwenstein-Jensen medium containing 500 and 1,000 γ of streptomycin per ml., 50 and 100 γ of viomycin per ml., and 0.1 and 1.0 γ of isoniazid per

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[9] Dihydrostreptomycin was always used instead of streptomycin.

[10] Koch's phenomenon is understood to be the local hypersensitive reaction at the sites of reinfection that tends to heal as a consequence of the immunity developed by the first infection. This is in contrast to the progressive ulceration at the sites of injection in animals not previously infected. It is known that the intensity of the local reaction and the involvement of the lymph nodes near the sites of reinfection will depend upon the size of the reinfecting inoculum as well as on other factors. As the second inoculum in the present experiments was large, an ulcerative and necrotizing reaction always occurred. The occurrence of Koch's phenomenon referred only to those animals in which the location reaction tended to heal.[11]

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TABLE 1

IDENTIFICATION OF BACILLI ISOLATED FROM ORGANS OF THE GUINEA PIGS INCLUDED IN THIS STUDY

Organs	Subgroups of Animals (*See* text)									
	A_1	A_2	B_1 and B_2	C_1	C_2	C_3	C_4	D_1 and D_2	E_1	E_2
Lymph nodes near the site of primary infection	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	—	—	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	Streptomycin-resistant	—†
Lymph nodes near the site of reinfection			Viomycin-resistant					Viomycin-resistant	Viomycin-resistant	Viomycin-resistant
Paravertebral lymph nodes	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	—*	—	—	—	Viomycin-resistant	Viomycin-resistant	—
Spleen	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	—‡	—	—	—	Viomycin-resistant	Viomycin-resistant	—
Liver	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	—	—	—	—	Viomycin-resistant	Viomycin-resistant	—
Lungs	Streptomycin-resistant	Viomycin-resistant	Streptomycin-resistant	—	—	—	—	Viomycin-resistant	—	—

* In one guinea pig two streptomycin-resistant colonies per tube were isolated.
† In one guinea pig some streptomycin-resistant colonies per tube were isolated.
‡ In one guinea pig three streptomycin-resistant colonies per tube were isolated.
— No bacilli could be isolated.

ml. Details of this technique have been reported elsewhere.[12]

Virulence of the streptomycin- and viomycin-resistant mutants of the H37Rv strain (Group A): Two groups of 8 guinea pigs each were inoculated with the streptomycin-resistant (*Subgroup* A_2) and viomycin-resistant (*Subgroup* A_2) strains, respectively. Two months later all animals were sacrificed. Each had a strong hypersensitive reaction to tuberculin.

The streptomycin-resistant strain was highly virulent. Widespread lesions were found by gross examination of internal organs, and extensive granulomas with central necrosis were found microscopically. Similar results were obtained with the parent streptomycin-susceptible strain. Drug-susceptibility tests demonstrated that the strains recovered from the organs of the streptomycin-resistant inoculated animals were resistant to 1,000 γ of streptomycin per ml.; no lessening of streptomycin resistance had occurred.

Although the viomycin-resistant strain could be isolated from all of the organs of guinea pigs inoculated with this strain, the lesions observed were not as extensive as those found in the organs of the animals inoculated with the streptomycin-resistant or parent strains. The viomycin-resistant strains recovered had the same degree of resistance as before inoculation. Despite the slight fall observed in the virulence of this strain, it was believed that it was suitable for the experiments.

Effect of reinfection with the viomycin-resistant strain on primary tuberculosis caused by the streptomycin-resistant strain (Group B): Twelve guinea pigs, each inoculated with the streptomycin-resistant strain, were reinfected two months later with the viomycin-resistant strain. Six animals were killed one month after reinfection (*Subgroup* B_1). The 6 animals remaining made up *Subgroup* B_2. Of these, one animal died at forty and another at forty-six days after reinfection. The remaining 4 animals were sacrificed two months after reinfection. Tuberculin reactions were strongly positive in all tested animals of both subgroups, and at autopsy the animals showed very far advanced tuberculosis with granulomatous lesions and large zones of necrotic tissue. After reinfection took place, a typical Koch's phenomenon was observed at the sites of reinfection. All cultures were positive for tubercle bacilli. The bacilli recovered from the organs were streptomycin resistant, except those isolated from sites of reinfection (with their respective regional lymph nodes) which were resistant to viomycin (table 1).

[12] BOJALIL, L. F., *et al.*: Acta tuberc. scandinav. (in press).

The influence of isoniazid on the streptomycin- and viomycin-resistant strains "in vivo" (Group C): Two groups of 12 guinea pigs each were infected with streptomycin- and viomycin-resistant strains, respectively. Treatment with isoniazid was started on the day of infection and was continued for a period of two months.

Six animals of each group (*Subgroups* C_1 and C_2 for the animals inoculated with the streptomycin- and viomycin-resistant strains, respectively) were sacrificed at the end of treatment. None showed evolutive lesions in their organs grossly or microscopically. Positive cultures were obtained from only 2 of these animals (infected with streptomycin-resistant strain). In one animal, two colonies per tube were recovered from paravertebral lymph nodes, and in the other, three colonies per tube were isolated from the spleen. Tuberculin tests became positive (++) one month after infection and were found to be the same when the animals were sacrificed.

The remaining animals (*Subgroups* C_3 and C_4 for the animals inoculated with the streptomycin- and viomycin-resistant strains, respectively) were sacrificed two months after isoniazid treatment was discontinued. These animals had stronger tuberculin reactions (+++ or ++++) than those sacrificed at the end of isoniazid treatment. All of the guinea pigs showed enlarged lymph nodes near the sites of infection. Focal granulomatous inflammation with no evidence of necrosis or giant cells was found in these nodes. No lesions were found in other organs. Cultures taken from the enlarged lymph nodes were positive for *M. tuberculosis*.

These findings strongly suggest that such treatment with isoniazid was adequate to arrest bacillary multiplication in the guinea pigs inoculated with the streptomycin- and viomycin-resistant mutants of human tubercle bacilli. Some multiplication could occur, however, after isoniazid treatment was stopped.

Effect of reinfection in arrested tuberculosis (Group D): In this experiment, 12 guinea pigs were infected with the streptomycin-resistant strain, and tuberculosis was arrested by means of isoniazid therapy as in *Group C*. After two months of treatment, the isoniazid administration was discontinued and all of the animals were challenged with the viomycin-resistant strain. Six guinea pigs were killed one month after reinfection (*Subgroup* D_1) and the remaining animals were sacrificed two months after reinfection (*Subgroup* D_2).

The internal organs of all of the animals showed moderately advanced tuberculous lesions by both gross and microscopic examinations. The animals of *Subgroup* D_2 had more severe lesions than those

of *Subgroup* D_1. Tuberculin tests were strongly positive. At the sites of reinfection, hard nodules were found. In some of the animals the nodules were ulcerated, with drainage of abundant purulent material. All of the cultures were positive for *M. tuberculosis*. The bacilli recovered were resistant to viomycin except those bacilli isolated from the inguinal lymph nodes (corresponding to the sites of primary infection) which were streptomycin resistant.

It was obvious that the second infection overcame the immune state produced by the first inoculation. The lesions observed in these animals, probably caused by the viomycin-resistant strain, were less severe than those found in the organs of the guinea pigs used as controls of virulence for the viomycin-resistant strain (*Subgroup* A_2). It is well known that the immune state reduces the severity of the infectious process in reinfected animals. The second infecting dose was, in effect, smaller than the first since it had to overcome the immune state induced by the primary infection.

Effect of isoniazid therapy on reinfected tubercle bacilli (*Group E*): The procedure followed in this group of 12 animals (*Group E*) was similar to that followed in *Group D*, except that isoniazid treatment was continued after reinfection. Six animals were sacrificed one month after reinfection (*Subgroup* E_1). They all showed considerable damage at the sites of reinfection, and the respective regional lymph nodes were found to be slightly enlarged, with focal granulomatous lesions in the corresponding tissue sections. No lesions were detected in other organs even though tubercle bacilli were isolated by culture. No bacilli could be isolated from the lungs of these animals.

The remaining animals of this group were sacrificed two months after reinfection (*Subgroup* E_2). No lesions were observed in the organs of these animals, and cultures were positive for *M. tuberculosis* only at the sites of reinfection and in the respective inguinal lymph nodes. In one guinea pig the site of primary infection was positive, the strain being streptomycin resistant; all other bacilli isolated were viomycin resistant.

The tuberculin reaction was strongly positive (++++) one month after reinfection. At this time the sites of reinfection showed hard nodules which, in some cases, were ulcerated. One month later the nodules had the same appearance, but in 2 animals they had almost completely disappeared.

* * *

The use of resistant mutants of tubercle bacilli as labelled strains for studies on reinfection has proved to be satisfactory, at least under the conditions of these experiments. This would confirm the work of Osato and associates.[7]

In a previous communication,[13] the present writers reported that after early treatment with isoniazid and streptomycin, tubercle bacilli of a single infection were restricted to the lymph nodes near the site of injection. The failure in finding tubercle bacilli in organs far from the sites of infection was observed even after drug treatment was discontinued. In this study, approximately the same results were obtained with isoniazid treatment only.

The present observations lead to the conclusion that the morphologic picture of the tissues in the animals of *Group D* has been altered by reinfection, since evolutive lesions were seen and reinfecting bacilli were isolated by culture from almost all of the organs of these animals. This is in contrast to the animals of *Subgroup* C_2 (primary infection only, isoniazid treatment for two months, sacrificed two months later) whose tissues did not show these morphologic changes.

When cultures for tubercle bacilli were obtained from the organs of reinfected guinea pigs, *mixtures of the inoculated strains were never found*. In the group of nontreated guinea pigs (*Group B*) the reinfection inoculum was restricted to the sites of injection and the lymph nodes surrounding these places; in the other organs pure cultures of the first injected strain were always isolated; perhaps the reinfecting strain neither multiplied nor disseminated. On the other hand, when the primary tuberculosis was arrested with isoniazid, the challenge infection not only was disseminated but also caused lesions in the guinea pigs. The primary injected strain was restricted to the sites of injection and to the corresponding lymph nodes. The possibility exists that in both cases reinfecting and primary infecting bacilli occurred simultaneously in the organs of the animals but they were so few that they were masked by the predominant strain.

Isoniazid therapy also had an effect against the reinfecting bacilli (*Group E*). However, its influence was not as pronounced when compared with its action against the same strain in a single infection (*Subgroup* C_2). These results are not in accord with those of Mitchison and Selkon,[14] who have emphasized that isoniazid can act synergistically with immunity against reinfecting tubercle bacilli. It is possible that the explanation of this discrepancy in results lies in the different experimental conditions employed.

The finding that those groups of guinea pigs which achieved the most effective control of the tuberculous infection also had the lowest degree of tuberculin sensitivity is in agreement with the

[13] Bojalil, L. F., *et al.*: Am. Rev. Tuberc., 1958, 77, 473.

observations of many investigators.[2, 4, 6, 13-16] The present study also confirms that an increase in degree of the tuberculin reaction occurs after discontinuation of isoniazid.

On the other hand, it has been suggested[3, 6, 14] that effective early isoniazid administration interferes indirectly with the development of immunity by inhibiting bacillary multiplication *in vivo* but only when the animals are infected with a small number of organisms. The present results can not be compared directly with those of these latter investigators, since, in the present studies, large numbers of bacilli were used for both primary infection and reinfection. Nevertheless, it was found that in the animals of *Group D* (treated, reinfected, isoniazid administration discontinued after reinfection), less severe lesions were observed than in the controls of virulence for the reinfecting strain (*Subgroup* A_2). Apparently the multiplication of the challenge infection was somewhat inhibited by the immunity produced by the first infection.

In summary: The effect of reinfection in guinea pig tuberculosis was followed by the use of streptomycin- and viomycin-resistant mutants of virulent tubercle bacilli. Both strains were shown to be: pathogenic for guinea pigs; susceptible to isoniazid; and susceptible to the alternate drug.

When guinea pigs were inoculated with either strain and isoniazid was administered for a two-month period from the day of infection, multiplication of both strains was inhibited. Two months after isoniazid therapy was discontinued, lesions appeared but they were restricted to the lymph nodes near the sites of infection.

Another group of animals was infected with the streptomycin-resistant strain and two months later the animals were reinfected with the viomycin-resistant strain. A typical Koch's phenomenon was observed, since the reinfecting strain was restricted to the site of injection. However, when the primary tuberculosis was arrested with isoniazid, the reinfecting strain in the absence of continuing isoniazid treatment was disseminated and caused lesions in the animals; the primary infecting strain was restricted to the sites of injection and the neighboring lymph nodes. Isoniazid therapy also was effective against the reinfecting bacilli.

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[14] MITCHISON, D. A., AND SELKON, J. B.: Am. Rev. Tubere., 1956, *74* (Supplement, p. 109).

[15] SCHMIDT, L. H.: Am. Rev. Tubere., 1956, *74* (Supplement, p. 138).

[16] GENTRY, W. H.: Am. Rev. Tubere., 1958, *77*, 940.

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SERUM ENZYMES IN PULMONARY TUBERCULOSIS: GLUTAMIC OXALACETIC TRANSAMINASE AND GLUTAMIC PYRUVIC TRANSAMINASE

In recent years the concept of biochemical determination of a number of the components of serum has been accepted and utilized extensively. Currently, a large group of serum enzymes are under investigation. It is hoped that some of these may be of value, not only in the diagnosis and prognosis of various pathologic conditions, but also in the elucidation of specific metabolic derangements essential to the disease state. Serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase are the two members of this group to which most attention has thus far been directed.

The oxalacetic transaminase catalyzes the reaction: alpha-keto glutarate + L-aspartate = glutamate + oxalacetate. The pyruvic transaminase catalyzes the reaction: alpha-keto glutarate + L-alanine = glutamate + pyruvate.

The study of alterations in the amount of these enzymes in serum is of considerable value, particularly in the diagnosis and prognosis of myocardial infarction and hepatic disease. Qualitatively, similar changes occur in various other disease states but are of less magnitude and specificity. Reports indicate that these enzymes are increased in a variety of conditions. The patients involved in such studies have frequently included some with active tuberculous infection. However, to the writers' knowledge, no report has yet appeared which deals exclusively with the oxalacetic transaminase and the pyruvic transaminase in active pulmonary tuberculosis or which includes a significant number of such patients. This study was initiated to determine the diagnostic and prognostic value of determinations of the oxalacetic transaminase and the pyruvic transaminase in pulmonary tuberculosis.

The values for the oxalacetic and pyruvic transaminases were determined with a Beckman D.U. spectrophotometer at 505 mμ with commercially available reagents. The oxalacetic transaminase was determined by both the Karmen and Frankel methods. Since the object of the study was to determine the value of oxalacetic and pyruvic transaminase determinations in pulmonary tuberculosis exclusively, all cases were excluded in which at least one condition was present which is usually or frequently associated with elevation of the value for these enzymes. With the exception of one patient with musculoskeletal trauma and another with recent myocardial infarction, those excluded had demonstrable liver disease. Patients found free of tuberculosis in any form were also excluded. Thus, the study which initially involved more than 500 determinations in approximately 250 patients eventually contained 98 patients with active pulmonary tuberculosis and 133 patients with inactive pulmonary tuberculosis. Activity and extent of disease were determined in accordance with the *Diagnostic Standards* of the National Tuberculosis Association.

Initially, it was planned to follow previously untreated active cases with periodic assays of their serum enzymes. However, it soon became apparent that such determinations were, from the first, within normal limits. The only exceptions to this were samples examined following an intercurrent episode of excessive alcohol ingestion. The latter was of sufficient constancy that unexpected elevations in oxalacetic and pyruvic transaminase prompted re-questioning of patients who denied recent alcoholic sprees. Almost invariably, they admitted such episodes when confronted with the findings.

The results of oxalacetic and pyruvic transaminase determinations in 98 cases of active pulmonary tuberculosis are presented in table 1. There was one patient who presented abnormal values for oxalacetic and pyruvic transaminase on three occasions. There was neither clinical nor laboratory evidence of cardiac or hepatic disease, and the patient repeatedly denied excessive alcohol ingestion. Numerical elevations of oxalacetic transaminase (Frankel method) or pyruvic transaminase were each noted in a single patient. In these 2 patients, the oxalacetic and pyruvic transaminase values were no more than 10 units above normal and could conceivably be attributed to laboratory variation. However, the first patient had oxalacetic transaminase concentrations greater than 100 units and pyruvic transaminase concentrations greater than 60 units over a period

TABLE 1

INCIDENCE OF ELEVATION OF OXALACETIC AND PYRUVIC TRANSAMINASE IN ACTIVE PULMONARY TUBERCULOSIS

		Oxalacetic Transaminase (Karmen Method)	Oxalacetic Transaminase (Frankel Method)	Pyruvic Transaminase
Minimal	18	0	1	1
Moderately advanced	43	0	0	1
Far advanced	37	0	1	0
	98	0	2	2

TABLE 2

INCIDENCE OF ELEVATION OF OXALACETIC AND PYRUVIC TRANSAMINASE IN INACTIVE PULMONARY TUBERCULOSIS

	Oxalacetic Transaminase (Karmen Method)	Oxalacetic Transaminase (Frankel Method)	Pyruvic Transaminase)
Minimal.....29	1	1	2
Moderately advanced...74	6	7	4
Far advanced.....29	3	3	2
133	10	11	8

of six weeks in spite of lack of demonstrable cause. Further follow-up study may reveal that he was in the incubation stage of hepatitis.

In table 2, the results of oxalacetic and pyruvic transaminase determinations in 133 patients with inactive pulmonary tuberculosis are indicated. There were 15 patients who had one or more elevated values. Elevation of 10 units or less occurred in 9 patients in whom no usual cause for much aberration could be found. The remaining 6 patients were chronic alcoholics who exhibited moderate increase of 10 to 50 units in serum enzymes and admitted recent prolonged debauches.

In summary: Comparison of the results of determination of oxalacetic and pyruvic transaminase in patients with active or inactive tuberculosis failed to reveal any significant difference with respect to alteration in such enzymes ascribed to pulmonary tuberculosis. Of interest was the frequency with which an alcoholic debauche was associated with mild or moderate transitory alteration in the value for these enzymes, often without consistent change in the standard liver function tests.

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A COMPARISON OF SPIROMETRIC AND DOUGLAS BAG MEASUREMENTS OF MAXIMAL BREATHING CAPACITY

The Douglas Bag and standard spirometers are widely used to record maximal breathing capacity. Shephard[1] has criticized the former, stating that special wide-necked bags and low-resistance valve boxes are needed for reliable results. On the other hand, Bartlett and Hecht[2] have demonstrated that a fourfold increase in expiratory airway resistance has little effect on the maximal breathing capacity, thereby eliminating the need for special low-resistance equipment. Shephard's additional criticisms, that the accuracy of Douglas Bag measurements depends upon split-second tap manipulation and that the final results give no indication of respiratory rate, are well warranted, but neither precludes accurate results.

Cinematographic analysis[3] of the spirometric procedure has illustrated that at certain stages during the maximal breathing capacity test the spirometer bell excursions are not recorded accurately by its pulley-connected writing pen. This suggests that the spirometric method may yield lower values than other more faithful methods of recording. In addition, the resistance and inertia associated with standard spirometric apparatus have prompted Bernstein and co-workers[4] to design a new spirometer and, for similar reasons, Shephard[5] studied the maximal breathing capacity with a pneumotachographic system.

Therefore, a comparison of Douglas Bag and spirometric measurements of the maximal breathing capacity of 20 healthy young men was undertaken to see if the inertia, resistance, and recording problems of a standard spirometer lowered the maximal breathing capacity sufficiently to warrant modifications in spirometer design or the use of other low-resistance apparatus. Any discrepancies between the two methods of measurement should be more at the upper range of maximal breathing capacity values. Hence, rather than use hospital patients, 20 young men, all of whom were engaged in regular athletic competition, were selected as subjects. Vital capacity measurements were also made for comparison of the maximal breathing capacity/vital capacity ratios with those of others using different types of apparatus. This index, as indicated by Shephard,[1] appears to be relatively independent of variations in surface areas, age, and test period respiratory rate.

All of the vital capacity measures were recorded with a standard 13.5-liter spirometer[6] (mouthpiece bore 22 mm.). All flutter valves and the soda lime container were removed from the apparatus. Records of the spirometer bell excursions were made on a kymograph (speed 32

TABLE 1

PHYSICAL CHARACTERISTICS AND RESPIRATORY MEASUREMENTS OF SUBJECTS

Subject	Height	Weight	Surface Area (M^2)	Vital Capacity	Maximal Breathing Capacity	
					Douglas Bag	Spirometer
	inches	*pounds*		*liters BTPS**	*(liters BTPS* per minute)*	
1	68	140	1.75	4.75	149	135
2†	68	148	1.79	4.42	150	148
3†	69	145	1.80	6.83	190	185
4†	69	143	1.80	5.15	177	190
5	70	145	1.81	4.82	188	173
6†	72	146	1.86	6.22	207	168
7	67	167	1.88	5.43	223	198
8	71	155	1.89	5.18	184	164
9	72	152	1.90	5.41	223	225
10†	72	159	1.93	5.96	183	172
11	73	157	1.94	5.67	132	139
12	72	163	1.96	5.74	235	244
13†	71	180	2.02	5.92	189	148
14	75	170	2.04	5.78	192	144
15	70	196	2.04	5.99	206	214
16	74	183	2.10	6.24	224	201
17†	74	195	2.16	5.77	254	223
18	74	199	2.17	5.17	170	180
19	74	200	2.17	7.21	249	246
20	75	235	2.32	6.21	211	192
Mean..	71.5	168.9	1.97	5.694	197	184
S.D....	2.5	24.1	0.13	0.67	32	33

Note: The mean age of the subjects was 20.6 years (standard deviation 0.28, range 19 to 27 years).

* Saturated with water vapor at ambient pressure.

† Subjects who attempted Douglas Bag test before spirometric measurement of maximal breathing capacity.

[1] SHEPHARD, R. J.: J. Physiol., 1957, *135*, 98.

[2] BARTLETT, R. E., JR., AND HECHT, H.: J. Appl. Physiol., 1957, *11*, 79.

[3] STEAD, W. S., *et al.*: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1957, p. 334.

[4] BERNSTEIN, L., *et al.*: Thorax, 1952, *7*, 255.

[5] SHEPHARD, R. J.: Thorax, 1955, *10*, 258.

[6] Manufactured by Wannen E. Collins Inc., Boston, Massachusetts.

TABLE 2

MEAN MAXIMAL BREATHING CAPACITY/VITAL CAPACITY RATIOS FOR HEALTHY MALE SUBJECTS (Age Range Twenty to Thirty*)

	Number of Patients	Maximal Breathing Capacity / Vital Capacity	Maximal Breathing Capacity Measuring Apparatus†	Subjects' Posture During Both Tests
Shephard	15	32.0	Pneumotachograph	Standing
Gilson and Hugh-Jones	10	29.8	8-liter spirometer without flutter valves or soda lime container	Sitting
Needham, *et al.*	27	29.8	100-liter Douglas Bag	Sitting
Matheson, *et al.*[10]	194	32.8 (Standard deviation 4.4)	Standard Benedict Roth spirometer without flutter valves or soda lime container	?
Baldwin, *et al.*[11]	17	31.4	9-liter spirometer without flutter valves or soda lime container	Standing
Present writer	20	34.7 (Standard deviation 4.9)	100-liter Douglas Bag	Standing
	20	32.5 (Standard deviation 5.4)	13.5-liter spirometer without flutter valves or soda lime container	Standing

* The age range for Baldwin, *et al.* was sixteen to thirty-four, mean, 25.5.
† Assumed all vital capacity measures by spirometer.

mm. per 12 seconds). The maximal breathing capacity was recorded using the ventilometer attachment to the same spirometer. The subjects also performed the maximal breathing capacity by exhaling into a previously evacuated Douglas Bag (mouthpiece bore 22 mm.) for a 12-second test period. The collected volumes were measured with a dry gas meter,[7] previously calibrated against a Tissot spirometer at a constant speed of evacuation.

Three standing vital capacity measurements were made with the closed circuit spirometer system. Three standing maximal breathing capacity tests were given with the spirometer and three with the Douglas Bag on the same day, with a time interval of two to five minutes allowed between each test. The best measurement of each test group was recorded and computed at 37°C., saturated with water vapor at ambient pressure. Seven subjects attempted the Douglas Bag tests first, the remainder beginning with the spirometer tests of maximal breathing capacity. Comparable respiratory rates were used in both methods during the 12-second test period.

The physical characteristics and respiratory measurements of the subjects are listed in table 1. The mean of the spirometric maximal breathing capacity values was 184.45 liters per minute, 6 per cent less than the Douglas Bag mean of 196.8 liters per minute. This mean 6 per cent difference was found to be significant at the 1 per cent level of confidence ($t = 3.01$), but does not appear to outweigh the additional advantages of spirometer measurements wherein a permanent record of the subject's tidal volume, respiration rate, and appearance of fatigue is obtained. Practice during the first method of maximal breathing capacity measurement did not result in a higher score's being recorded for the second method (*see* table 1).

The maximal breathing capacity/vital capacity ratios as determined by other investigators using a variety of apparatus are compared in table 2. Shephard[1] has used such a comparison to conclude that a low-resistance pneumotachographic system records higher results for maximal breathing capacity than either the spirometer used by Gilson and Hugh-Jones[8] or the Douglas Bag used by Needham.[9] However, Shephard's subjects were standing for the maximal breathing capacity,[5] whereas the subjects in the other studies were sitting. The sitting position tends to restrict the maximal breathing capacity more than the vital capacity; hence the maximal breathing capacity/vital capacity ratio is lower than an index based on standing measurements. The difficulty of upholding Shephard's conclusion is further illustrated in the light of the higher ratios cited for

[7] Manufactured by the American Meter Company, Albany, New York.

[8] GILSON, J. C., AND HUGH-JONES, P.: Special Report Series, Medical Research Council, London, 1955, No. 290.

[9] NEEDHAM, C. D., *et al.*: Thorax, 1954, *9*, 313.

[10] MATHESON, H. W., *et al.*: J. Clin. Invest., 1950, *29*, 682.

[11] BALDWIN, E., *et al.*: Medicine, 1948, *27*, 243.

both spirometric and Douglas Bag methods in this study. It was believed that these two high ratios might have been due to the athletic nature of the subjects, but recently the index was computed for 20 nonathletic medical students using the Douglas Bag method, and an even higher mean maximal breathing capacity/vital capacity ratio of 36.6 (standard deviation 5.1) was established. Clearly, the use of special low-resistance apparatus cannot be justified in terms of this ratio.

In summary: The maximal breathing capacities of 20 young men were measured with a 13.5-liter spirometer and a 100-liter Douglas Bag. The mean Douglas Bag measurement was 6 per cent higher than the mean spirometric measurement; this difference is statistically significant at the 1 per cent level of confidence, but it is concluded that the additional advantages of the standard spirometer outweigh this slight discrepancy.

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Books

ANNUAL REVIEW OF MEDICINE, Vol. 9. Edited by David A. Rytand. Pp. 530, Annual Reviews, Inc., Palo Alto, California, 1958. Price, $7.00.

This volume, like its predecessors in this series, is a collection of reviews of the relatively recent literature pertaining to certain selected areas within the scope of internal medicine and to some fields which, while not strictly within the internist's usual purview, are nevertheless of interest to him in a biologic and clinical sense. Included in the latter group are surveys of recent advances in dentistry, psychiatry, plastic surgery (including homotransplantation), and pediatrics. There is a timely and rational review of the new discipline of psychopharmacology which is refreshing in its physiologic and pharmacologic orientation. In addition, Lasagna and Meier have contributed a brief but sound essay on clinical evaluation of drugs.

The above-mentioned sections are presented in the volume in addition to competent reviews in the more classical subdivisions of medicine, such as gastroenterology, cardiovascular disease, infections, diseases of the kidney, hematology, endocrinology, immunology, and so on.

Although it is impossible for a single volume to be truly a review of all of medicine's advances in a year, the present volume is composed of a good and representative selection of important work in most of the major medical disciplines in the past year or two. The individual pieces are by and large well-written, and, as is usually the case, the *Annual Review of Medicine* provides a reliable and engrossing key to the recent medical literature.

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TRACHEOTOMY: A CLINICAL AND EXPERIMENTAL STUDY. By Thomas G. Nelson. Pp. 111, The Williams & Wilkins Company, Baltimore, 1958. Price $3.75.

Thomas G. Nelson's book on tracheotomy is essentially a review which, despite the addition of new clinical material, adds little to our present knowledge of the subject. Nevertheless, it is on the whole a fairly comprehensive work which should be of interest to every practioner of medicine, particularly to those whose practice necessitates thorough understanding of this field, e.g., the surgeon, anesthesiologist, and obstetrician.

After briefly describing the historical development of this operation, the author presents a fairly detailed discussion of its indications, the technique of the procedure, its possible complications, and the results in the 310 tracheotomies observed in a fairly large and representative series of 310 patients with mechanical ventilatory or secretional ventilatory obstruction. This material occupies about two-thirds of the 111-page volume.

The remaining one-third is devoted to experimental work with animals. The purpose of the study was to test the effect of various factors in the development of either laryngeal or tracheal stenosis after tracheotomy. The author states that the experimental work includes what appears to be the first study of stenosis after relatively long-term healing. His findings should be of more than routine interest to workers in this field.

This reviewer is in agreement with the author's conclusion that all tracheotomies should be performed through a transverse incision. Emphasis is properly placed on the dangers of a delay in performing this operation when it is clearly indicated. Those lacking background or experience in this procedure may find this book quite valuable as a means of acquiring the detailed information needed for prompt decision and action.

The abundant illustrations of clinical and experimental material and autopsy specimens are excellent and will be particularly helpful to those whose experience with this operation is limited.

It is the opinion of the reviewer that, while this book will be of interest to all who may be called upon to perform a tracheotomy, its contents are fairly familiar to every practicing surgeon. To assure maximal exposure, perhaps the more important aspects of this material might better have been presented in a medical journal rather than in a book.

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ANTIBIOTICS ANNUAL 1957–1958. Edited by Henry Welch and Felix Marti-Ibanez. Pp. 1070, Medical Encyclopedia, Inc., New York, 1958. Price $12.00.

The *Antibiotics Annual 1957–1958* is a compilation of papers and panel discussions presented at the Fifth Annual Symposium on Antibiotics held in October, 1957, in Washington, D.C. The papers cover a wide range of subjects, including chemistry, pharmacology, microbiologic studies, therapeutic evaluation in animals, and clinical studies on a variety of new and older antibiotics; relation

of antibiotics to nutrition; veterinary applications of antibiotics; antibiotics in the treatment of plant diseases; food preservation with antimicrobials; complications of antibiotic therapy and their management; use of steroids as adjuncts in the treatment of infections; effects of antibiotics on normal host flora; antibiotic combinations; antitumor antibiotics; and natural resistance to infections.

The panel discussions are "Rheumatic Fever Prophylaxis," "Host Resistance and Chemotherapy," and "Antibiotics as Anti-tumor and Antiviral Substances."

In addition, there is the first William Randall Lecture delivered by Ernest Jawetz; this lecture, entitled "Patient, Doctor, Drug, and Bug" is an excellent over-all discussion on the rational use of antibiotics and factors determining the likelihood of success following their use.

Although this book can be criticized on the basis of inclusion of a number of substandard articles, there is enough outstanding material on antibiotics and infectious diseases to make the over-all result a valuable work. It will be of particular interest to those concerned with infectious diseases and with the administration of antibiotics in clinical medicine and to the pharmacologist; but should serve as a reference source for people in all areas of medicine and related fields.

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Los Angeles, California

BOOKS RECEIVED

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American Trudeau Society

Medical Section of the National Tuberculosis Association

MANUAL FOR CONSECUTIVE CASE CONFERENCE[1]

(Pembine Type)

Prepared for the American Trudeau Society Committee on Medical Education by
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The Committee on Therapy of the American Trudeau Society has prepared this *Manual for Consecutive Case Conferences* to assist any groups who are interested in setting up a conference similar to the original Pembine Conference and to provide general information concerning such a conference. The material in the *Manual* is presented in considerable detail for those who are organizing their first consecutive case conference. Each section is concerned with some one phase of planning and organizing.

Part I is devoted to the Pembine Conference, its historical background and methods.

Part II states the objectives of a consecutive case conference. Basically, these are the presentation of consecutive cases and a critical evaluation of the procedures used in treatment.

Part III is concerned with the planning of a conference. Subjects presented include the general organization of the steering committee, methods of financing, selection of those attending and participating, planning and developing the program, and the selection of the cases to be presented.

Part IV outlines the facilities that may contribute to a successful conference.

Part V describes the manner in which a conference is conducted.

Part VI discusses the means by which the information gained at the conference can be made available to others.

I. HISTORY AND DEVELOPMENT OF THE PEMBINE CONFERENCE

In 1940, the State of Michigan appointed a Sanatorium Survey Committee to visit all the sanatoriums in the state periodically and to review in detail the cases under treatment at the time of the visit. The cases were presented on a consecutive admission basis to ensure that all patients were reviewed by the Committee. A report was submitted to the hospital superintendent and, subsequently, to the Michigan Department of Health. Later, a similar committee plan was adopted in Wisconsin. It became the practice in both states to invite tuberculosis specialists and sanatorium directors in the vicinity to attend these survey meetings. It was soon apparent that differences in policies and treatment existed between the sanatoriums within a state and, to a greater extent, between the states. The Pembine Conference idea was born in a discussion of this point at one of these meetings by representatives from the three states of Michigan, Wisconsin, and Minnesota.

The first conference, known as the Michigan-Wisconsin-Minnesota Regional Tuberculosis Therapy Conference, was held at the Four Seasons Club, a private resort hotel, on June 3 and 4, 1944. This meeting was sponsored by the American Trudeau Society, and part of the expenses were defrayed by the Society. A report of the Conference was published in the AMERICAN REVIEW OF TUBERCULOSIS in December, 1944.

There was uncertainty at the time whether the conferences could and should be continued. The enthusiasm engendered by the initial conference was so great, however, that a second conference was held in 1945 at the same location. The conference, renamed the Pembine[2] Conference in 1946, became an annual affair, with a regular date of the first week-end after Labor Day.

II. OBJECTIVES

A Pembine type of conference has a principle so interrelated with the method that it would be almost impossible to separate one from the other.

The purpose is to *generate a critical review and evaluation* of the therapies and procedures used. This is accomplished by the *presentation of consecutive cases*. Such a method permits comparative

[1] Copies of this manual can be obtained from the American Trudeau Society, 1790 Broadway, New York 19, New York.

[2] The Four Seasons Club is a private resort hotel located approximately fourteen miles south and east of Pembine, Wisconsin. The Club is on an island in the Menominee River, the boundary between Wisconsin and Michigan. Those attending the early conferences traveled by train to the nearest railroad station and were taken by private cars to the Club. "Going to Pembine" became synonymous with attending the Therapy Conference.

evaluations of all cases within a particular series and also contributes to a better comparison between two or more parallel series. The pattern of consecutive cases ensures that *no selection* has been exercised by the presenter and that, within the series, both the unfavorable and the good results must appear. If a sufficient number of cases is presented, the policies of the hospital in regard to treatment and the adequacy of the therapy planning will become apparent.

The facts in the case under discussion are inescapably recorded in the roentgenograms and in the clinical data presented. Anyone offering a criticism must be prepared to defend his own position in an adequate manner and to support it by facts. A critical evaluation of therapies and procedures requires a frank, free, and friendly discussion. It must be recognized and accepted that the purpose of such discussions is *to criticize ideas, concepts, procedures, techniques, and even attitudes,* but *not institutions or persons.*

This concept of the critical review of consecutive cases has some interesting and important potentials. The method has been used most extensively and quite effectively as an educational medium. It may be used for the demonstration of any disease or condition which can be presented in this manner. It is possible to demonstrate the results that are obtained with some specified type of therapy. Thus it may be useful to evaluate some newer form of therapy. These types of presentation have their drawbacks. The results are assessed too frequently on a relatively short-term observation period. Some particular type of therapy can be popularized, especially if the presenter is convincing. It is difficult to reach any conclusions as to the superiority of one form of therapy over another.

This leads then into the next possible use for this method of presenting cases—as a research study method whereby *evaluations are made from a long-term observation period.* This was done at a Conference when a series of pneumothorax cases was reviewed again after a period of ten years. A similar follow-up review of consecutive admissions after an interval of years undoubtedly would be most revealing. The major problem would be the difficulty in locating all of the patients.

The second phase is to establish criteria for the selection of subjects. Too stringent requirements can eliminate many factors that should be considered, whereas too broad a subject may result in the critical points being lost in extraneous data. This can be avoided by an objective approach. First, learn the facts about the conference area: the most divergent opinions, the greatest disagreements, the greatest needs, the fields of greatest interest. A subject can be selected from these. Then, two general questions may be applied: *What is the problem to be demonstrated? What is the question to be answered?* The consecutive cases used should be those which bring out these points more clearly, or best exhibit the features to be discussed.

The cases are not to be considered as individual reviews. They form the background for the subject under discussion. Total admissions will demonstrate the multiple problems that the staff must meet, while total discharges will show the end results actually attained. How patients admitted for the first time are handled will serve one purpose, whereas a study of readmission cases, particularly if good social data are available to stress the human side of patients, will be enlightening in another way. Specific therapies can be reviewed for both the unfavorable and the favorable results, or, by shifting the starting or cut-off dates, can be studies for the immediate or the long-range results.

The immediate objective, then, is to learn the facts as they actually exist, select from this a problem of major concern, and then make a critical evaluation of the problem from a background of adequate and representative cases.

There are a few general objectives which will contribute to the attainment of the primary purposes:

Some degree of *informality* will lead to freer discussions. The direction of the conference should be effective but, at the same time, it should be unobtrusive.

The basic discussions will be a *critical evaluation* of *therapies* and *procedure*; however, comments of a pertinent nature and arising from some point in the case under discussion will be introduced. These comments are equally informative and add much. The moderator must determine the time that can be allowed without infringing upon the presentations.

This type of conference is not designed to mold or determine opinions. Rather, it contemplates that each one attending will observe the facts as presented in the case reviews, will evaluate the criticisms made, and then will come to his own conclusions of what is adequate and effective. More important, the critical methods of the conference should be extended to staff meetings, with an equally critical reappraisal of the therapies and procedures being employed.

III. PLANNING A CONFERENCE

No one plan for a consecutive case conference will be applicable to every meeting; nor can one expect to duplicate exactly such a conference in several localities. Each conference must be

planned to fit into the community or area which it will serve. The first and very essential step is to determine the actual potentials in a conference area. This should include consideration of the hospitals that will participate, the number and professional quality of those who may attend and, perhaps, just how much interest there may be for holding such a conference. The plans for a conference can and should be adapted to stay within the bounds of the potentials as they actually exist. Adjustments can be made which will provide for the optimal use of the facilities and materials that are available.

Planning committee: There must be some individual or group who will assume the initiative for planning the conference. This sponsorship usually will be within the area concerned. The impetus may come from someone who has attended a consecutive case conference in another section of the country. A hospital in the area may take the responsibility, but it should be understood that the hospital is providing only the basic facilities. Certainly, it must avoid the criticism that the hospital staff is trying to promote or publicize its own treatments and procedures. Existing organized groups interested in tuberculosis work may serve as sponsors.

The steering, or executive, committee which directs the activities of the conference should be representative of the conference area. The division or pattern of representation will be determined locally. Usually a small committee is more effective. The success of the conferences will depend upon this committee. It is essential, therefore, that the members be chosen with care and thought. The committee, as constituted, has many responsibilities and must resolve many problems. It prepares the agenda for the conference, selects the guests and issues the invitations to them, and makes the local arrangements for the meeting. It needs, and should have, full authority to act on all matters pertaining to the conference.

Finances: The financing of a conference may be a problem, even though expenses are held to a minimum. Tuberculosis associations or Trudeau societies are supporting many of these conferences. A small registration fee will help. The extent of the expenses incurred and the manner in which they will be paid must be determined locally. Special guests invited to the conference may expect some remuneration to cover necessary expenses.

It is advisable for each of the conference members to make his own financial arrangements. A person attending at his own expense has more than average interest in the conference or he would not accept the invitation.

Selection of participants: Members should be selected from hospitals, clinics, and private practice to obtain a well-balanced cross-section of the conference area. The group should include the specialists: pathologists, radiologists, thoracic surgeons, and internists. Representatives should come from small hospitals and communities as well as from the larger ones.

The conference should be held to a size that can ensure adequate inspection of the roentgenograms by all present. It will usually be found that approximately 50 members is the maximum. This type of conference does not require or lend itself to large audiences.

Date and location of conference: A date for holding the conference will depend upon local preferences and available accommodations. Some resort clubs, hotels, or motels can be reserved exclusively before or at the end of their normal season.

The conference location and surroundings may contribute materially to its success. The primary objective should be to keep the group as intact as possible and in surroundings that will encourage frequent and close contact between the members.

Program planning: The cases presented for comparative evaluation must have some elements in common: consecutive admissions, consecutive discharges, consecutive surgical cases, or consecutive cases treated by some specified type of therapy. The starting or cut-off *dates should be uniform and apply equally to all series.* It is well to avoid any period during which major changes in therapy occurred, since this will affect the comparative value of the series. If a new therapy is being considered, then some allowances must be made for the readjustment period within the hospitals and for the influence that experience has had on the selection of cases.

Formal papers and presentations should be used sparingly and cautiously. Any such presentation should be relevant to the case subjects and of interest to all persons attending.

Timing: A time schedule is essential for the general chairman and for the moderators. The moderator can watch the time and use it as a control over general discussions from the floor. The first series of cases presented usually stimulates the major portion of the discussion, and the initial presenter takes a greater share of the general criticism. Ample time should be allowed for the discussions that will arise. Six to eight cases per hour is about the maximum if discussion is lively. If the time allotted each presenter is insufficient, then cases should be eliminated rather than have restrictions placed on the discussions. The moderator may ask if there are particular cases remain-

ing which illustrate some point. These can be used to close the presentation.

Individual case summary: Some form of outline guide or summarization of the clinical data on each patient is very essential. The format and pertinent information to be included can be determined by the steering committee. These outlines are prepared in mimeographed or printed form by the hospital presenting the cases. One set should be available to each conferee, with additional sets available for record purposes or to send to interested groups. Graphic outlines have the advantage in that information is available at a glance and the relationships between various therapies can be diagrammed. Narrative or text charts provide more specific information.

Instructions: Instructions sent by the steering committee to the presenters should be concise and explicit. These should include the methods for selecting cases, the preparation of the outline summaries, information about roentgenograms and about auxiliary equipment.

The display of roentgenograms is a most important feature. A few pertinent films will tell the story better than a long series of films. These films may be supplemented by special films, such as planigrams or contrast media films, to demonstrate a significant point. Dates in large figures, near the bottom of the film, will assist those in the back of the audience.

The steering committee should send to each member and guest any instructions and general information that might be of assistance. Data concerning rail and air connections will be helpful to many. Highway data should be included for those traveling by private automobile. The time for the meetings should be specified and, if possible, the agenda for the conference should be made available prior to the actual conference date.

Correspondence: All correspondence should be centralized in one person, preferably the chairman of the steering committee. It would be well to set up a correspondence file and to maintain copies of all letters, at least temporarily. It will be found that some of the correspondence contains material for setting up policies for future conferences. Any significant correspondence should become part of the archives of the conference.

IV. CONFERENCE FACILITIES AND ARRANGEMENTS

Registration: The registration desk can distribute the general programs of the conference and the roster of those in attendance. The roster, giving the names and home addresses of all members attending, assists the group in getting acquainted with each other. Identifying name tags also help. The registration fee can be paid at the registration desk unless other arrangements are made.

Assembly room: The conference room should provide for the comfort of those attending and for good visibility. The size of the room should be compatible with the number attending. Movable chairs permit the conferees to adjust themselves for better viewing of the roentgenograms and closer grouping about the view boxes. The room should be just dark enough for good visualization of films or of projected material, and still permit reading of the case summaries.

Visual aids and equipment: The equipment required will vary with the type of conference and with the material presented. Six to eight roentgenographic view boxes in a single row are better than a double bank. View boxes should be elevated (five feet is a good height) for unobstructed vision from the back rows and should be located for optimal viewing. Low-power binoculars aid in viewing films from a distance. A roentgenographic projector can be used to project and enlarge selected films and particularly to display films smaller than 14 by 17 inches. Projection facilities should be available for both 35 mm. and 3¼- by 4-inch lantern slides. A projector screen set to one side of the view boxes permits a rapid change from roentgenograms to slides. Also, one film can be projected while others are left in the view boxes and illuminated. A microscope is essential for the pathologists if tissue slides are reviewed. A blackboard, with chalk and eraser available, may be used for illustrating points in the discussion. Pointers and indicators for use with the films or with projected material help to identify some particular feature without obstructing the screen or view boxes.

Recording the conferences: Amplifying equipment is unnecessary in a small room. Recording equipment may be used. Direct transcription requires considerable work to transcribe and to edit the information recorded. A brief running summary of presentations and discussions can be kept in longhand by a designated recorder. This form of summarization will be adequate for most occasions.

Distribution of outlines: The case outline summaries should be distributed just before each new presentation. This permits a break between the series of cases and gives the new presenter an opportunity to arrange his material and roentgenograms. The outlines are available at the right time and place.

Visitors: The casual or uninvited visitor is a matter to be handled by the steering committee or by a general conference policy. The facilities available may determine whether such visitors

can be accepted. The invited conferees must be given priority with respect to the seating.

V. CONDUCT OF CONFERENCE AND CASE PRESENTATIONS

Direction of conferences: The *chairman* is responsible for the over-all conduct of the conference. He maintains liaison with the groups presenting cases and serves as the intermediary between the conference and the hotel management. He has the responsibility for seeing that the general time schedules are maintained. A *moderator* is in immediate charge of each separate presentation or session. This moderator may be one of the general committee members, or he may be some member of the conference group designated to act in this capacity. The moderator should not be an associate of the presenter, nor should he have been concerned in any way with the treatment of the group of patients under discussion.

Presentation of cases: An initial and final summation by the presenter will help in the evaluation of the cases. The preliminary summarization should include facts about the hospital and patients: the general source of patients, the admission policy, information regarding the staff and hospital facilities. The closing summation should give data concerning the cases presented. This might include the stages of disease represented, the therapies used, and the results obtained.

The case reviews require adequate preparation. The presenter must be thoroughly familiar with the cases and should use notes for reference only. If possible, the presenter should have been responsible directly for the care and general management of the patients while they were in the hospital. An assistant should handle all roentgenograms. This leaves the presenter and the moderator free to participate in the discussions. The presenter should arrange with the projectionist as to the timing of any projected material.

Discussions: The discussion from the floor must be frank, friendly but, above all, *critical*. The moderator will have full direction and control of any and all discussions. He must be prepared to hold to pertinent subjects and be ready to close discussions at an appropriate time. He may encourage participation by referring questions to selected members. Directed questions may be necessary at the start of the conference to bring capable but reticent conferees into the discussions. They may be used also to develop the views of younger men. The moderator should keep the cases moving as rapidly as possible, with due consideration for the need for discussion.

Free and critical discussions are sometimes more important than cases. The case reports and reviews are intended to stimulate this discussion. The presenter should be ready to delete cases if time becomes a factor, but he should do this only at the direction of the moderator. Critical discussions usually will continue in informal groups after the meetings.

Summation of conference: The conference should close with a brief business session. Visiting guests may be invited to offer critical observations relative to the conference as a whole. If this is the initial conference, the steering committee may wish to learn the opinions of the members and their wishes for subsequent conferences. Written comments on recommendations and programs may be solicited. More attention may be given such letters, since they are prepared after some thought and deliberation.

VI. FOLLOW-UP OF CONFERENCE

Since the consecutive case conference serves as an educational medium, the information derived should be made available to those who have been unable to attend. This can be achieved through review conferences and publications.

One objective of this type of conference is that those who attend will, in turn, transmit to their fellow staff members or to others in their group the information gained at the conference. It is advisable at times to choose the conferees with this point in mind. The conferences in general are not designed, nor are they able, to prove that one treatment is superior to another. They are designed to stimulate and encourage a willingness to evaluate therapies and procedures on their individual merits and a desire to consider in an equally critical manner the results obtained or hoped for in any specific cases.

A published summary of the proceedings of the conference is quite valuable. It will provide a uniform source of reference for those attending and give them a guide for later discussions in their own groups. Such proceedings should contain a general review of the cases presented, a brief review of pertinent discussion concerning cases or of general discussions growing out of the cases under review. Comments made by visiting guests at the close of the conference might be included.

The committee should consider submitting a brief résumé of the conference for publication in state or national journals. This review should be concise and limited to general conclusions or to the most significant discussions. A list of those attending as special guests, or those who were invited to make special contributions to the conferences, can add some prestige to the conference

Acceptance for publication may depend upon the caliber and the scope of the conference.

The committee should take steps immediately after the first conference to develop an historic archive for the organization. The value of these archives increases as the conferences are continued. The good features of any conference deserve a permanent record. Their historic value may not be apparent for some time after the conference.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29

1960—Los Angeles, California—May 15–20

FOURTH INTERNATIONAL COURSE ON TOMOGRAPHY

September–October, 1959

The Fourth International Course on Tomography will be held some time during September–October, 1959, at the Radiology Institute, University of Genoa, Italy, under the direction of Professor Alessandro Vallebona.

Further details may be obtained from Professor Neopolo Macarini, General Secretary, Istituto di Radiologia della Universita, Ospedale San Martino, Genova, Italia.